

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : G01N 33/68, 33/573, 33/74	A1	(11) International Publication Number: WO 99/23493 (43) International Publication Date: 14 May 1999 (14.05.99)
(21) International Application Number: PCT/US98/22797 (22) International Filing Date: 27 October 1998 (27.10.98) (30) Priority Data: 08/961,809 31 October 1997 (31.10.97) US 09/178,691 26 October 1998 (26.10.98) US (71) Applicant: THE ROCKEFELLER UNIVERSITY [US/US]; 1230 York Avenue, New York, NY 10021-6399 (US). (72) Inventors: LI, Cai; Apartment #15D, 500 East 63rd Street, New York, NY 10021 (US). FRIEDMAN, Jeffrey, M.; Apartment #6C, 151 Central Park West, New York, NY 10023 (US). (74) Agents: DAVIS, Michael, D. et al.; Klauber & Jackson, 411 Hackensack Avenue, Hackensack, NJ 07601 (US).		(81) Designated States: CA, JP, MX, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: METHODS OF IDENTIFYING AGENTS THAT MODULATE LEPTIN ACTIVITY		
(57) Abstract The present invention discloses novel methods for identifying drugs that can help regulate adiposity and fat content of animals, particularly in mammals. The discovery that PTP-1D binds to the phosphorylated leptin receptor when the receptor contains a phosphorylated tyrosine-985, provides a novel means for identifying agents to aid in the regulation of body weight and adiposity. Thus the present invention exploits this prior unknown role of PTP-1D by providing means for potentially treating and curing abnormalities of the endogenous leptin pathway, as well as allowing for the elected modification of body mass.		

ATTORNEY DOCKET NUMBER: 9142-006-999
SERIAL NUMBER: 09/489,873
REFERENCE: CJ

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

METHODS OF IDENTIFYING AGENTS THAT MODULATE LEPTIN ACTIVITY

GOVERNMENTAL SUPPORT

5 The research leading to the present invention was supported, at least in part, by a grant from National Institute of Health, Grant No. DK41096. Accordingly, the Government may have certain rights in the invention.

FIELD OF THE INVENTION

10 The present invention relates to methods for identifying agents that can modulate the effect of the signal transduction pathway initiated by the natural hormone, leptin. Such agents can be used to aid the modulation of mammalian body mass. In particular, a new member of this signal transduction pathway has been identified. This new member, PTP-1D, has previously been identified as being a protein tyrosine phosphatase. The present invention discloses that the modulation of the phosphorylation state of PTP-1D can have an important effect on the biological activity of leptin. Therefore, the present invention has important therapeutic implications in homeostasis of body weight and fat tissue mass.

BACKGROUND OF THE INVENTION

20 Obesity, defined as an excess of body fat relative to lean body mass, is associated with important psychological and medical morbidities, the latter including hypertension, elevated blood lipids, and Type II or non-insulin-dependent diabetes melitis (NIDDM). There are 6-10 million individuals with NIDDM in the U.S., including 18% of the population of 65 years of age [Harris *et al.*, *Int. J. Obes.*, 11:275-283 (1987)]. Approximately 45% of males and 70% of females with NIDDM are obese, and their diabetes is substantially improved or eliminated by weight reduction [Harris, 25 *Diabetes Care*, 14(3):639-648 (1991)]. Both obesity and NIDDM are strongly

heritable, and the predisposing genes within the relevant controlled regulatory pathways are just beginning to be identified.

The assimilation, storage, and utilization of nutrient energy constitute a complex homeostatic system central to survival of metazoa. Among land-dwelling mammals, storage in adipose tissue of large quantities of metabolic fuel as triglycerides is crucial for surviving periods of food deprivation. The need to maintain a fixed level of energy stores without continual alterations in the size and shape of the organism requires the achievement of a balance between energy intake and expenditure. The identification of the relevant factors in the complex homeostasis system and the identification of agents that can be administered to control these factors is therefore of great importance.

An individual's level of adiposity is, to a large extent, genetically determined. Examination of the concordance rates of body weight and adiposity amongst mono- and dizygous twins or adoptees and their biological parents have suggested that the heritability of obesity (0.4-0.8) exceeds that of many other traits commonly thought to have a substantial genetic component, such as schizophrenia, alcoholism, and atherosclerosis [Stunkard *et al.*, *N. Engl. J. Med.*, 322:1483-1487 (1990)]. Familial similarities in rates of energy expenditure have also been reported [Bogardus *et al.*, *Diabetes*, 35:1-5 (1986)]. Genetic analysis in geographically delimited populations has suggested that a relatively small number of genes may account for the 30-50% of variance in body composition [Moll *et al.*, *Am. J. Hum. Genet.*, 49:1243-1255 (1991)]. However, none of the genes responsible for obesity in the general population have been genetically mapped to a definite chromosomal location.

The most intensively studied mouse obesity mutations are the *ob* (obese) and *db* (diabetes) genes. Mice homozygous for either mutation are hyperphagic and hypometabolic, leading to an obese phenotype that is notable at one month of age. The weight of these animals tends to stabilize at 60-70 g (compared with 30-35 g in control mice).

Each of the rodent obesity models is accompanied by alterations in carbohydrate metabolism resembling those in Type II diabetes in man. In some cases, the severity of the diabetes depends in part on the background mouse strain [Leiter, *Endocrinology*, 124:912-922 (1989)]. For both *ob* and *db*, congenic C57BL/Ks mice develop a severe diabetes with ultimate β cell necrosis and islet atrophy, resulting in a relative insulinopenia. Conversely, congenic C57BL/6J *ob* and *db* mice develop a transient insulin-resistant diabetes that is eventually compensated by β cell hypertrophy resembling human Type II diabetes.

The phenotype of *ob* and *db* mice resembles human obesity in ways other than the development of diabetes - the mutant mice eat more and expend less energy than do lean controls (as do obese humans). This phenotype is also quite similar to that seen in animals with lesions of the ventromedial hypothalamus, which suggests that both mutations may interfere with the ability to properly integrate or respond to nutritional information within the central nervous system.

A major advance in understanding the molecular basis for obesity occurred with the cloning of the *ob* gene. The mouse obesity (*ob*) gene encodes an adipose tissue-derived signaling factor for body weight homeostasis [Zhang *et al.*, *Nature*, 372:425 (1994); U.S. Patent Application No. 08/292,345 filed August 1, 1994; U.S. Patent Application No. 08/483,211, filed June 7, 1995, each of which is hereby incorporated by reference in its entirety]. The recombinant OB protein (leptin) purified from *Escherichia coli* can correct the obesity related phenotypes in *ob/ob* mice when exogenously administered [Campfield *et al.*, *Science*, 269:546 (1995); Pellymounter *et al.*, *Science*, 269:540 (1995); Halaas *et al.*, *Science*, 269:543 (1995); Stephens *et al.*, *Nature*, 377:530 (1995)]. Weight-reducing effects of the recombinant leptin were also observed in normal mice and mice with diet-induced obesity. Another advance occurred with the determination that the *db* gene encoded the leptin receptor [U.S. Patent Application No. 08/586,594 filed January 16, 1996; U.S. Patent Application No. 08/783,734 filed January 16, 1997; and Tartaglia *et al.*, *Cell*, 83:1263-1271 (1995) each of which is hereby incorporated by reference in its entirety].

Current studies suggest that obese humans and rodents (other than *ob/ob* mice) are not defective in their ability to produce leptin mRNA or protein and generally produce higher levels than lean individuals [Maffei *et al.*, *Nature Med.*, 1:1155 (1995); Considine *et al.*, *J. Clin. Invest.*, 95:2986 (1995); Lonnqvist *et al.*, *Nature Med.*, 1:950 (1995); Hamilton *et al.*, *Nature Med.*, 1:953 (1995)]. These data suggest that resistance to normal or elongated levels of leptin may be important factors in human obesity.

Leptin is a 16kD hormone that is the afferent signal in a negative feedback loop regulating food intake and body weight. The leptin receptor is a member of the cytokine family. Leptin's anorexigenic effect is dependent on binding to the Ob-Rb isoform of this receptor which encodes a long intracytoplasmic domain that includes several motifs for protein-protein interaction. Ob-Rb is highly expressed in the hypothalamus suggesting that this brain region is an important site of leptin action. Signal transduction by this class of receptor generally depends on ligand induced phosphorylation of soluble tyrosine receptor kinases such as JAK1, 2, 3, and tyk2. These kinases in turn phosphorylate tyrosine residues on the receptor which serve as docking sites for SH2 proteins. Phosphorylation of SH2 proteins after receptor binding initiates signal transduction. Leptin binds to a homodimer of the Ob-Rb isoform of its receptor thus activating JAK2. While the Stat3 transcription is activated by leptin *in vivo*, the identity of other components of this signal transduction pathway have not yet been identified.

Therefore there is a need to identify the components of the endogenous leptin pathway, *i.e.* the participants in the transduction of the signal initiated by the binding of the leptin to the leptin receptor. Furthermore, there is a need to design assays that can be used to identify agents that can modulate the endogenous leptin pathway by modulating the identified component.

The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

SUMMARY OF THE INVENTION

The present invention provides a method of identifying a modulator of the binding of a phosphorylated leptin receptor with PTP-1D. One such embodiment comprises contacting the phosphorylated leptin receptor or phosphorylated fragment thereof with PTP-1D or fragment thereof in the presence and absence of an agent under conditions in which in the absence of the agent, the binding of the phosphorylated leptin receptor or phosphorylated fragment with PTP-1D or fragment thereof can be detected. The binding of the phosphorylated leptin receptor or phosphorylated fragment thereof with PTP-1D or fragment thereof is then detected. When an increase in binding is detected in the presence of the agent, the agent is identified as a modulator that enhances the binding of the phosphorylated leptin receptor or phosphorylated fragment thereof with PTP-1D or fragment thereof. Alternatively, when a decrease in binding is detected in the presence of the agent, the agent is identified as a modulator that inhibits the binding of the phosphorylated leptin receptor or phosphorylated fragment thereof with PTP-1D or fragment thereof. In embodiments such as this one, the phosphorylated leptin receptor or phosphorylated fragment thereof contains a phosphorylated tyrosine-985, *i.e.*, the leptin receptor or the fragment thereof contains a tyrosine which is analogous to tyrosine 985 of SEQ ID NO:2 which is phosphorylated and therefore is a binding partner of PTP-1D.

In one such embodiment the phosphorylated leptin receptor or phosphorylated fragment thereof is bound to a solid support. In another such embodiment PTP-1D or fragment thereof is labeled. In still another embodiment the phosphorylated leptin receptor or phosphorylated fragment thereof is bound to a solid support and PTP-1D or fragment thereof is labeled. The present invention also includes analogous embodiments in which the phosphorylated leptin receptor or phosphorylated fragment thereof is labeled; and/or the PTP-1D or fragment thereof is bound to a solid support. As is exemplified below the phosphorylated fragment can also be part of a fusion (or chimeric) protein. In a preferred embodiment of this type the fusion protein comprises a phosphorylated fragment of the leptin receptor fused to a

glutathione-S-transferase. In another preferred embodiment of this type the fusion protein comprises a phosphorylated fragment of the leptin receptor fused to green fluorescent protein.

Another aspect of the present invention includes methods of identifying a modulator for the phosphorylated leptin receptor-dependent PTP-1D phosphorylation. One such embodiment comprises contacting a phosphorylated leptin receptor or phosphorylated fragment thereof with PTP-1D and JAK2 kinase in the presence and absence of an agent under conditions in which in the absence of the agent the phosphorylated leptin receptor or phosphorylated fragment thereof can stimulate the phosphorylation of PTP-1D. In such embodiments the phosphorylated leptin receptor or phosphorylated fragment thereof contains a phosphorylated tyrosine-985. Next the amount of phosphorylation of PTP-1D is determined. When either an increase or decrease in the phosphorylation is determined in the presence of the agent, relative to in its absence, the agent is identified as a potential modulator of the leptin receptor-dependent phosphorylation of PTP-1D. This embodiment can include the subsequent step of contacting of the potential modulator with PTP-1D under the initial conditions of contacting (*i.e.*, the first step) except a phosphorylated leptin receptor or phosphorylated fragment thereof containing the phosphorylated tyrosine-985 is not included. When no significant change in phosphorylation is determined in the presence of the potential modulator relative to in its absence, the potential modulator is identified as a modulator of the leptin-dependent phosphorylation of PTP-1D. In a preferred embodiment of this type, the phosphorylated fragment is part of a fusion protein.

In one such embodiment the fusion protein comprises the phosphorylated fragment fused to a glutathione-S-transferase. In another embodiment the fusion protein comprises the phosphorylated fragment fused to green fluorescent protein.

Another aspect of the present invention includes methods of identifying a potential modulator and/or modulator of the leptin-dependent PTP-1D phosphorylation *in situ*.

One such embodiment comprises contacting a cell with leptin in the presence and absence of an agent under conditions in which in the absence of the agent leptin induces the phosphorylation of PTP-1D. In such embodiments the cell comprises PTP-1D, JAK2, and a leptin receptor which contains tyrosine-985. The amount of phosphorylation of PTP-1D is then determined. When an increase or decrease in phosphorylation of PTP-1D is determined in the presence of the agent relative to its absence, the agent is identified as a potential modulator of the leptin-dependent phosphorylation of PTP-1D.

In one such embodiment the cell is transfected with a vector which encodes either PTP-1D, JAK2, and a leptin receptor which contains tyrosine-985. In another embodiment the vector encodes two or more of the proteins. Alternatively, nucleic acids encoding two or more the proteins are transfected into the cell in two or more vectors individually encoding a protein. In a preferred embodiment the cell is transfected with a vector encoding PTP-1D, a vector encoding JAK2, and a vector encoding a leptin receptor which contains tyrosine-985.

In a preferred embodiment the method further comprises contacting a second cell with leptin and the potential modulator under the conditions of those of the initial step except in this case leptin cannot induce the phosphorylation of PTP-1D. The second cell comprises PTP-1D, JAK2, and a leptin receptor that does not contain tyrosine-985. The amount of phosphorylation of PTP-1D is then determined. When no significant change in phosphorylation is determined in the presence of the potential modulator relative to its absence the potential modulator is identified as modulator of the leptin-dependent phosphorylation of PTP-1D.

In one particular embodiment the leptin receptor that does not contain tyrosine-985 is the Ob-Ra form of the receptor. In another particular embodiment the leptin receptor that does not contain tyrosine-985 is the Ob-Rb containing a phenylalanine-985 as exemplified below.

In one such embodiment the cell is transfected with a vector which encodes either PTP-1D, JAK2, and a leptin receptor which does not contain tyrosine-985. In another embodiment the vector encodes two or more of the proteins. Alternatively, nucleic acids encoding two or more the proteins are transfected into the cell in two or more vectors individually encoding a protein. In a preferred embodiment the cell is
5 transfected with a vector encoding PTP-1D, a vector encoding JAK2, and a vector encoding a leptin receptor which does not contain tyrosine-985.

In one particular embodiment the modulator enhances the leptin receptor-dependent phosphorylation of PTP-1D. In another particular embodiment the modulator inhibits
10 the leptin receptor-dependent phosphorylation of PTP-1D.

The present invention further provides a method of identifying a drug useful in a weight loss diet regimen. One such embodiment comprises administering one or more doses, (preferably multiple doses) to a test animal of an agent found to be an inhibitory modulator of the leptin-dependent phosphorylation of PTP-1D and a
15 identical number of doses of a placebo to a control animal over a prescribed time period. The change in weight of the test animal and the control animal during the prescribed time period is then determined. An inhibitory modulator that causes the test animal to lose weight relative to the control animal is selected as a drug that is useful in a weight loss diet regimen.

20 The present invention further provides a method of identifying a drug useful in a weight gain diet regimen. One such embodiment comprises administering one or more doses, (preferably multiple doses) to a test animal of an agent found to be an enhancing modulator of the leptin-dependent phosphorylation of PTP-1D and a identical number of doses of a placebo to a control animal over a prescribed time
25 period. The change in weight of the test animal and the control animal during the prescribed time period is then determined. An enhancing modulator that causes the test animal to gain weight relative to the control animal is selected as a drug that is useful in a weight gain diet regimen.

In either embodiment, the prescribed time period for laboratory animals being the test animal is preferably one week to two years, more preferably one month to one year, and even more preferably two months to six months.

5 Still another variation of the present invention includes a method of identifying a modulator of the leptin-dependent PTP-1D dephosphorylation of the JAK2 kinase *in situ*. One such embodiment comprises contacting a cell with leptin in the presence and absence of an agent under conditions in which in the absence of the agent, leptin induces the PTP-1D dephosphorylation of JAK2. The cell comprises PTP-1D, JAK2, and a leptin receptor which contains tyrosine-985. Next, the amount of
10 phosphorylation of JAK2 is determined. When a decrease or increase in phosphorylation of JAK2 is determined in the presence of the agent relative to in its absence, the agent is identified as a potential modulator of the leptin-dependent PTP-1D dephosphorylation of JAK2. Then the cell is contacted with the potential modulator under the conditions of the initial step except in the absence of leptin.
15 When no significant change in phosphorylation is determined in the presence of the potential modulator relative to in the absence of the potential modulator, the potential modulator is identified as a modulator of the leptin-dependent PTP-1D dephosphorylation of JAK2.

20 Again in one such embodiment the cell is transfected with a vector which encodes either PTP-1D, JAK2, and a leptin receptor which contains tyrosine-985. In another embodiment the vector encodes two or more of the proteins. Alternatively, nucleic acids encoding two or more the proteins are transfected into the cell in two or more vectors individually encoding a protein. In a preferred embodiment the cell is transfected with a vector encoding PTP-1D, a vector encoding JAK2, and a vector
25 encoding a leptin receptor which contains tyrosine-985.

The present invention further includes a method of identifying an inhibitor of the leptin-dependent PTP-1D phosphorylation *in situ*. One such embodiment comprises (a)contacting a cell with leptin in the presence and absence of an agent under

conditions in which in the absence of the agent, leptin induces the expression of a reporter gene operably under the control of a promoter containing a binding site for activated Stat3. (b) The level of expression of the reporter gene contained by a host cell is determined. In this embodiment, the binding of activated Stat3 protein to the binding site induces the expression of the reporter gene in the cell comprises PTP-1D, JAK2, Stat3 and a leptin receptor which contains tyrosine-985. When the presence of the agent results in an increase in the level of expression of the reporter gene relative to in its absence, the agent is identified as a candidate inhibitor of the leptin-dependent PTP-1D phosphorylation. (c) Next, a second cell is contacted with leptin in the presence and absence of the candidate inhibitor under conditions in which in the absence of the candidate inhibitor leptin induces the phosphorylation of PTP-1D and wherein the second cell comprises PTP-1D, JAK2, and a leptin receptor which contains tyrosine-985. (d) The amount of phosphorylation of PTP-1D of step (c) is then determined. When a decrease in phosphorylation of PTP-1D is determined in the presence of the candidate inhibitor relative to in the absence of the candidate inhibitor, the candidate inhibitor is identified as a potential inhibitor of the leptin-dependent phosphorylation of PTP-1D. (e) A third cell is then contacted with leptin and the inhibitor under the conditions of those of step (c) except in this case leptin cannot induce the phosphorylation of PTP-1D. The third cell comprises PTP-1D, JAK2, and a leptin receptor that does not contain tyrosine-985. (f) The amount of phosphorylation of PTP-1D of step (e) then determined. When no decrease in phosphorylation is determined in the presence of the potential inhibitor relative to in its absence, the potential inhibitor is identified as an inhibitor of leptin-dependent phosphorylation of PTP-1D. Again the various proteins used in this assay can be native to the cell or preferably added by transfection of the cell with vectors encoding these proteins as described above.

In a preferred embodiment of this method, the reporter gene encodes luciferase. In a related embodiment the reporter gene encodes green fluorescent protein.

One aspect of this method further includes identifying a drug useful in a weight loss diet regimen. One such embodiment comprises administering one or more doses, (preferably multiple doses) to a test animal of an agent found to be an inhibitory modulator of the leptin-dependent phosphorylation of PTP-1D and a identical number of doses of a placebo to a control animal over a prescribed time period. The change in weight of the test animal and the control animal during the prescribed time period is then determined. An inhibitory modulator that causes the test animal to lose weight relative to the control animal is selected as a drug that is useful in a weight loss diet regimen.

The present invention further provides a method of identifying a drug useful in a weight gain diet regimen. One such embodiment comprises administering one or more doses, (preferably multiple doses) to a test animal of an agent found to be an enhancing modulator of the leptin-dependent phosphorylation of PTP-1D and a identical number of doses of a placebo to a control animal over a prescribed time period. The change in weight of the test animal and the control animal during the prescribed time period is then determined. An enhancing modulator that causes the test animal to gain weight relative to the control animal is selected as a drug that is useful in a weight gain diet regimen.

In either embodiment, the prescribed time period for laboratory animals being the test animal is preferably one week to two years, more preferably one month to one year, and even more preferably two months to six months.

These and other aspects of the present invention will be better appreciated by reference to the following drawings and Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Binding of a 64kD protein to phosphotyrosine-985 of the Ob-Rb cytoplasmic region. Figure 1A is a schematic representation of the full length leptin

receptor (Ob-Rb) and shows the three segments used to make the three Glutathione-S-Transferase (GST) fusion fragments. The three GST-fragments are ObRbTyr1, ObRbTyr2 and ObRbTyr3 which are named for the reversibly phosphorylatable tyrosines of this region: Tyrosine-985, Tyrosine-1077, and Tyrosine-1138 respectively.

Figure 1B shows the GST fusion fragments of the peptides spanning each of the three cytoplasmic tyrosines (Y 985, 1077, 1138) which were expressed in bacteria with or without co-expression of the elk tyrosine kinase. Co-expression of elk tyrosine kinase led to the specific phosphorylation of these three tyrosine residues. The GST-Ob-Rb fragment 1 peptide (containing Y985) was incubated with protein extracts from bovine or mouse hypothalamus. After precipitation with anti-GST antibodies, the bound proteins were eluted, resolved on SDS PAGE, and stained with Coomassie Blue. A 64kD protein was found in the precipitates of mouse and bovine hypothalamus. The 64kD protein was not precipitated by the anti-GST antibody when tyrosine 985 was not phosphorylated. The 64kD protein was sequenced and found to be identical to Phosphotyrosine Phosphatase ID.

Figure 2: Immunoblots of the protein precipitate of GST-ObRb fragments. Figure 2A depicts an immunoblot using anti-PTP-1D antibody. Figure 2B depicts an immunoblot using anti-Stat3 antibody. The phosphorylated and unphosphorylated GST-ObRb fusion fragments 1 and 3 were incubated with protein extracts of mouse hypothalamus. PTP-1D was detected only in the material precipitated by the phosphorylated form of GST-ObRbTyr1. Stat3 was detected only in the proteins precipitated by the phosphorylated form of GST-ObRbTyr3.

Figure 3: Binding of Leptin to Ob-Rb leads to the Inducible Phosphorylation of PTP-1D. 293 cells were transfected using combinations of Ob-R, JAK2 and PTP-1D as indicated. The state of PTP-1D phosphorylation was assayed with and without leptin treatment. PTP-1D was first assayed by immunoprecipitation using an anti PTP-1D antibody followed by immunoblotting using an anti-phosphotyrosine antibody. In

separate studies the total cell lysate was immunoblotted with the anti-phosphotyrosine antibody. Treatment of cells with leptin transfected with Ob-Rb, JAK2 and PTP-1D led to an increase PTP-1D phosphorylation. This was confirmed using either method of detection. Transfection of Ob-Ra, JAK2 and PTP-1D led to the constitutive phosphorylation of PTP-1D but the level of phosphorylation was unchanged by leptin treatment (a and b indicated Ob-Ra and Ob-Rb respectively).

Figure 4: PTP-1D Phosphorylation by Ob-Rb is Dependent on Tyr 985. 293 cells were transfected with wild type Ob-Rb or a Y→F 985 (*i.e.*, tyrosine to the phenylalanine substitution) mutant leptin receptor together with JAK2 and PTP-1D. The level of PTP-1D phosphorylation was scored at various times after leptin treatment. Leptin increased the level of PTP-1D phosphorylation only in the cells that received the wild type leptin receptor. PTP-1D was not phosphorylated in cells transfected with the Y→F 985 mutant.

Figure 5: Phosphorylated PTP-1D Dephosphorylates JAK2. The level of phosphorylation of JAK2 was assayed after leptin treatment in cells transfected with the wild type and Y→F 985 mutant. After leptin treatment, the level of JAK2 phosphorylation was five-fold greater in cells that received the wild type leptin receptor. When PTP-1D was cotransfected, the level of JAK2 phosphorylation was ten-fold greater in the cells that were transfected with the Y→F 985 mutant leptin receptor. These data indicated that binding of leptin to Ob-Rb phosphorylation of PTP-1D leads to the dephosphorylation of JAK2.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel assays for agents which can potentially serve as drugs in the modulation of mammalian body mass. The present invention is based in part on the discovery that Protein Tyrosine Phosphatase 1D, PTP-1D, described in United States Patent No. 5,589,375 hereby incorporated by reference in its entirety, is involved in the endogenous leptin pathway. More specifically, PTP-1D has been

found to bind to the phosphorylated leptin receptor, when the receptor contains a phosphorylated tyrosine-985.

The discovery of the role of PTP-1D has important implications for the treatment of nutritional disorders including, but not limited to, obesity, weight loss associated with cancer and AIDS and the treatment of diseases associated with obesity such as hypertension, heart disease, and Type II diabetes. In addition, there are potential agricultural uses for the agents identified by the methods described herein in cases where one might wish to modulate the body weight of domestic animals.

Therefore, if appearing herein, the following terms shall have the definitions set out below

As used herein, "physiological obesity" and "physiologically obese" refer to excessive adipose tissue that is due at least in part to abnormalities in the endogenous leptin pathway, including abnormalities in the effective signaling initiated by the binding of leptin to the leptin receptor. Abnormalities in the endogenous leptin pathway may be manifested in a number of ways including an abnormal food intake, an abnormal activity level, or an abnormal body temperature. In addition, the present invention allows drugs to be identified which can modulate body mass completely independently of any inherent abnormality in the endogenous leptin pathway per se by augmenting or diminishing the natural effect of leptin.

As used herein, "leptin" encompasses biologically active variants of naturally occurring leptin, as well as biologically active fragments of naturally occurring leptin and variants thereof, and combinations of the preceding. Leptin is the polypeptide product of the *ob* gene as described in the International Patent Publication No. WO 96/05309, and the US Patent Application No. 08/483,211 to which it claims priority, each of which is incorporated herein by reference in its entirety. Putative analogs and fragments of leptin are reported in US Patent 5,521,283, US Patent 5,532,336 and

International Patent Publication No. PCT 96/22308 for International Application No. PCT/US96/ 01471, each of which is incorporated herein by reference in its entirety.

As used herein the terms "bound" or "binds" or "associates" or "associated" are meant to include all such specific interactions that result in two or more molecules showing a preference for one another relative to some third molecule. This includes processes such as covalent, ionic, hydrophobic and hydrogen bonding but does not include non-specific associations such solvent preferences.

As used herein, the phrase "conditions related to abnormalities of the endogenous leptin pathway" encompasses conditions and diseases due, at least in part, to abnormalities involving leptin as detailed above.

The term "medically assisting" is used herein as a manner of attending to the health care needs of a subject who has a particular problem (*e.g.*, an abnormality in the endogenous leptin pathway) which encompasses either diagnosing or treating that problem, and all combinations thereof. In one embodiment, the invention provides for medically assisting a mammalian subject suffering from an abnormality in the endogenous leptin pathway resulting in decreased leptin activity. In another embodiment, a mammalian subject may be suffering from an abnormality resulting in increased leptin activity. In each case, the decreased or increased leptin activity may be manifested as a pathological state, such as obesity (decreased leptin activity) or anorexia (increased leptin activity).

The term "detectable" is used broadly herein to include a factor that directly emits the detectable signal (such as a fluorescent molecule) an entity bound directly to that factor (such as an antibody carrying a fluorescent molecule) as well as a detectable target of the factor or the entity (such as an epitope of an antigen that reacts with the antibody carrying a fluorescent molecule). Thus a target may be termed detectable due to either its potential or realized association with either a factor that functions as a detectable label or a factor that is bound to a detectable label. Detectable labels,

include but not limited to an enzyme, a radioactive element, a biochemiluminescent, a chromophore that absorbs in the ultraviolet and/or visible and/or infrared region of the electromagnetic spectrum; and a fluorophore.

5 As defined herein, a "quantitative relationship" between two or more determinations of one or more substances, includes the relative absolute amounts, a relative percentage, a relative ratio, the difference, sum, multiple and/or quotient for two or more determinations; and can further include appropriate first or higher order equations that express the relationship between two or more determinations in a manner that can be understood by a person skilled in the art to which the present
10 invention pertains.

A "determination" or "determining" as used herein is the result of an assay that includes an attempt to detect and/or the quantifying of the substance detected such as that expressed in a quantity or an amount of that substance.

15 As used herein the term "mono-specific antibody" describes an antibody to a protein raised against a particular peptide sequence in that protein and is meant to imply that the antibody is specific for an epitope that includes that particular sequence. A monospecific antibody may be either polyclonal or monoclonal.

20 As used herein a phosphorylated leptin receptor or phosphorylated fragment thereof contains a phosphorylated "tyrosine-985" or "Y985" when the receptor or fragment thereof contains the amino acid sequence of Amino Acid 983 to Amino Acid 990 of SEQ ID NO:2 and tyrosine-985 is phosphorylated. In a preferred embodiment, the receptor or fragment thereof contains the amino acid sequence of Amino Acid 971 to Amino Acid 1000 of SEQ ID NO:2 and tyrosine-985 is phosphorylated. In a more
25 preferred embodiment, the receptor or fragment thereof contains the amino acid sequence of Amino Acid 961 to Amino Acid 1010 of SEQ ID NO:2 and tyrosine-985 is phosphorylated. As exemplified below, the fragment contained Amino Acid 965 to Amino Acid 1001 of SEQ ID NO:2 and tyrosine-985 is phosphorylated.

In analogous embodiments, a leptin receptor can contain a dephosphorylated tyrosine-985 (*i.e.*, a tyrosine that is not phosphorylated at position 985 of SEQ ID NO:2) or a phenylalanine at position 985 of SEQ ID NO:2 in place of the tyrosine. As used herein, the receptor containing such a substitution is referred to "F985" or the "F985 mutant receptor."

The OB receptor contains three important structural domains: an extracellular (or extracytoplasmic) domain, a transmembrane domain, and a cytoplasmic domain. The extracellular domain binds leptin. The transmembrane domain comprises a stretch of highly non-polar amino acid residues that localize to the hydrophobic region of the cell membrane. In this respect, the term transmembrane domain has its ordinary meaning in molecular and cellular biology. Finally, the cytoplasmic domain of an OB receptor of the invention may contain none, one, or two JAK-binding consensus sequences, termed "Box 1" and "Box 2". A receptor having "Box 1" and "Box 2" is believed competent for signal transduction via the JAK-Stat pathway upon binding of leptin.

In a further aspect, the OB-R polypeptide from one species is closely related (homologous) to the OB-R in another species. In particular, the human OB-R polypeptide is highly homologous to murine OB-R polypeptide. This observation is consistent with the data showing that human leptin is active in mice: for the hormone to be active interspecies, one would expect a high degree of similarity or homology between the receptors from different species as well.

In its primary aspect, the present invention is directed to the identification of agents that function as modulators of mammalian body weight.

The term "substantially similar" as used herein means at least 50% sequence similarity, preferably at least 60% sequence similarity, more preferably at least 70% sequence similarity, even more preferably at least 80% sequence similarity, and most preferably at least 90% sequence similarity.

The term "gene" as used herein refers to a nucleic acid, such as DNA, which codes on expression for a protein. Unless stated otherwise, gene may include mRNA, cDNA, or genomic DNA.

5 A composition comprising "A" (where "A" is a single protein, DNA molecule, vector, recombinant host cell, etc.) is substantially free of "B" (where "B" comprises one or more contaminating proteins, DNA molecules, vectors, etc., but excluding racemic forms of A) when at least about 75% by weight of the proteins, DNA, vectors (depending on the category of species to which A and B belong) in the composition is "A". Preferably, "A" comprises at least about 90% by weight of the A+B species in
10 the composition, most preferably at least about 99% by weight. It is also preferred that a composition, which is substantially free of contamination, contain only a single molecular weight species having the activity or characteristic of the species of interest.

15 Contemplated by the present invention are analogs comprising conservative amino acid substitutions. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity, which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include
20 alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. In some instances, one polar amino
25 acid may be substituted with another to preserve local hydrophilicity; more likely, a substitution that conserves charge, or at least does not introduce the opposite charge, is required. Such alterations will not be expected to affect apparent molecular weight as determined by polyacrylamide gel electrophoresis, or isoelectric point.

Particularly preferred substitutions are:

- Lys for Arg and vice versa such that a positive charge may be maintained;
- Glu for Asp and vice versa such that a negative charge may be maintained;
- Ser for Thr such that a free -OH can be maintained; and
- 5 - Gln for Asn such that a free NH₂ can be maintained.

Amino acid substitutions may also be introduced to substitute an amino acid with a particularly preferable property. For example, a Cys may be introduced a potential site for disulfide bridges with another Cys. A His may be introduced as a particularly "catalytic" site (i.e., His can act as an acid or base and is the most common amino acid
10 in biochemical catalysis). Pro may be introduced because of its particularly planar structure, which induces β -turns in the protein's structure.

As used herein, the term "homologous" in all its grammatical forms refers to the relationship between proteins that possess a "common evolutionary origin," including proteins from superfamilies (e.g., the immunoglobulin superfamily) and homologous
15 proteins from different species (e.g., myosin light chain, etc.) (Reeck et al., 1987, Cell 50:667). Such proteins have sequence homology as reflected by their high degree of sequence similarity.

Accordingly, the term "sequence similarity" in all its grammatical forms refers to the degree of identity or correspondence between nucleic acid or amino acid sequences of
20 proteins that may or may not share a common evolutionary origin (*see* Reeck et al., *supra*). However, in common usage and in the instant application, the term "homologous," when modified with an adverb such as "highly," may refer to sequence similarity and not a common evolutionary origin.

The term "corresponding to" is used herein to refer similar or homologous sequences,
25 whether the exact position is identical or different from the molecule to which the similarity or homology is measured. Thus, the term "corresponding to" refers to the

sequence similarity, and not the numbering of the amino acid residues or nucleotide bases.

A gene encoding the leptin receptor, leptin, PTP-1D or JAK2, whether genomic DNA or cDNA, can be isolated from any animal source, particularly from a mammal.

5 Methods for obtaining the *Stat protein* gene are well known in the art, as described above (*see, e.g.,* Sambrook *et al.*, 1989, *supra*).

A "heterologous nucleotide sequence" as used herein is a nucleotide sequence that is added to a nucleotide sequence of the present invention by recombinant methods to form a nucleic acid which is not naturally formed in nature. Such nucleic acids can
10 encode chimeric and/or fusion proteins. Thus the heterologous nucleotide sequence can encode peptides and/or proteins which contain regulatory and/or structural properties. In another such embodiment the heterologous nucleotide can encode a protein or peptide that functions as a means of detecting the protein or peptide encoded by the nucleotide sequence of the present invention after the recombinant
15 nucleic acid is expressed. In still another such embodiment the heterologous nucleotide can function as a means of detecting a nucleotide sequence of the present invention. A heterologous nucleotide sequence can comprise non-coding sequences including restriction sites, regulatory sites, promoters and the like.

Nucleic Acids

20 In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. *See, e.g.,* Sambrook *et al., Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989); Glover ed., *DNA Cloning:*
25 *A Practical Approach*, Volumes I and II, MRL Press, Ltd., Oxford, U.K. (1985); Gait ed., *Oligonucleotide Synthesis*, Oxford University Press (1984); Hames *et al.*, eds., *Nucleic Acid Hybridization*, Springer-Verlag (1985); Hames *et al.*, eds. *Transcription And Translation*, Oxford University Press (1984); Freshney ed., *Animal Cell Culture*,

Oxford University Press (1986); *Immobilized Cells And Enzymes*, IRL Press (1986); Perbal, *A Practical Guide To Molecular Cloning*, Wiley, New York (1984). Of particular relevance to the present invention are strategies for isolating, cloning, sequencing, analyzing, and characterizing a gene or nucleic acid based on the well known polymerase chain reaction (PCR) techniques.

A "replicon" is any genetic element (*e.g.*, plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*, *i.e.*, capable of replication under its own control.

A "vector" is a replicon, such as a plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "cassette" refers to a segment of DNA that can be inserted into a vector at specific restriction sites. The segment of DNA encodes a polypeptide of interest, and the cassette and restriction sites are designed to ensure insertion of the cassette in the proper reading frame for transcription and translation.

A cell has been "transfected" by exogenous or heterologous DNA when such DNA has been introduced inside the cell.

A "clone" is a population of cells derived from a single cell or common ancestor by mitosis.

A "nucleic acid molecule" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules") in either single-stranded form, or a double-stranded helix. Double-stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit

it to any particular tertiary or quaternary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (e.g., restriction fragments), plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation.

A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single-stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (*see* Sambrook *et al.*, 1989, *supra*). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a T_m of 55°C, can be used, e.g., 5x SSC, 0.1% SDS, 0.25% milk, and no formamide; or 30% formamide, 5x SSC, 0.5% SDS). Moderate stringency hybridization conditions correspond to a higher T_m , e.g., 40% formamide, with 5x or 6x SCC. High stringency hybridization conditions correspond to the highest T_m , e.g., 50% formamide, 5x or 6x SCC. Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T_m) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived (*see* Sambrook *et al.*, 1989, *supra*, 9.50-0.51). For hybridization with shorter nucleic acids, *i.e.*, oligonucleotides, the position of mismatches becomes

more important, and the length of the oligonucleotide determines its specificity (*see* Sambrook *et al.*, 1989, *supra*, 11.7-11.8). Preferably a minimum length for a hybridizable nucleic acid is at least about 18 nucleotides; more preferably at least about 27 nucleotides; most preferably the length is at least about 36 nucleotides.

- 5 In a specific embodiment, the term "standard hybridization conditions" refers to a T_m of 55°C, using conditions as set forth above. In a preferred embodiment, the T_m is 60°C, in a more preferred embodiment, the T_m is 60°C.

- 10 "Homologous recombination" refers to the insertion of a foreign DNA sequence of a vector in a chromosome. Preferably, the vector targets a specific chromosomal site for homologous recombination. For specific homologous recombination, the vector will contain sufficiently long regions of homology to sequences of the chromosome to allow complementary binding and incorporation of the vector into the chromosome. Longer regions of homology, and greater degrees of sequence similarity, may increase the efficiency of homologous recombination.

- 15 A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide in a cell *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not
20 limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (*e.g.*, mammalian) DNA, and even synthetic DNA sequences. If the coding sequence is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

- 25 Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the

expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences.

A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then trans-RNA spliced and translated into the protein encoded by the coding sequence.

A DNA sequence is "operatively linked" to an expression control sequence when the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (*e.g.*, ATG) in front of the DNA sequence to be expressed and maintaining the correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene that one desires to insert into a recombinant DNA molecule does not contain an appropriate start signal, such a start signal can be inserted upstream (5') of and in reading frame with the gene.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase.

25

Antibodies

According to the invention, leptin receptor, PTP-1D, or the various proteins used in the fusion fragments described herein can be produced recombinantly or by chemical

synthesis, and may be used as an immunogen to generate antibodies that recognize the proteins. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library.

5 A molecule is "antigenic" when it is capable of specifically interacting with an antigen recognition molecule of the immune system, such as an immunoglobulin (antibody) or T cell antigen receptor. An antigenic polypeptide contains at least about 5, and preferably at least about 10, amino acids. An antigenic portion of a molecule can be that portion that is immunodominant for antibody or T cell receptor recognition, or it can be a portion used to generate an antibody to the molecule by conjugating the
10 antigenic portion to a carrier molecule for immunization. A molecule that is antigenic need not be itself immunogenic, *i.e.*, capable of eliciting an immune response without a carrier.

An "antibody" is any immunoglobulin, including antibodies and fragments thereof, that binds a specific epitope. The term encompasses polyclonal, monoclonal, and
15 chimeric antibodies, the last mentioned described in further detail in U.S. Patent Nos. 4,816,397 and 4,816,567, as well as antigen binding portions of antibodies, including Fab, F(ab')₂ and F(v) (including single chain antibodies). Accordingly, the phrase "antibody molecule" in its various grammatical forms as used herein contemplates both an intact immunoglobulin molecule and an immunologically active portion of an
20 immunoglobulin molecule containing the antibody combining site. An "antibody combining site" is that structural portion of an antibody molecule comprised of heavy and light chain variable and hypervariable regions that specifically binds antigen.

Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule
25 that contains the paratope, including those portions known in the art as Fab, Fab', F(ab')₂ and F(v), which portions are preferred for use in the therapeutic methods described herein.

Fab and F(ab')₂ portions of antibody molecules are prepared by the proteolytic reaction of papain and pepsin, respectively, on substantially intact antibody molecules by methods that are well-known. See for example, U.S. Patent No. 4,342,566 to Theofilopolous *et al.* Fab' antibody molecule portions are also well-known and are
5 produced from F(ab')₂ portions followed by reduction of the disulfide bonds linking the two heavy chain portions as with mercaptoethanol, and followed by alkylation of the resulting protein mercaptan with a reagent such as iodoacetamide. An antibody containing intact antibody molecules is preferred herein.

The phrase "monoclonal antibody" in its various grammatical forms refers to an
10 antibody having only one species of antibody combining site capable of immunoreacting with a particular antigen. A monoclonal antibody thus typically displays a single binding affinity for any antigen with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule having a plurality of antibody combining sites, each immunospecific for a different antigen; *e.g.*, a
15 bispecific (chimeric) monoclonal antibody.

The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. An adjuvant can serve as a tissue depot that slowly releases the antigen and also as a lymphoid system activator that non-specifically enhances the immune response [Hood *et al.*, in *Immunology*, p. 384, Second Ed.,
20 Benjamin/Cummings, Menlo Park, California (1984)]. Often, a primary challenge with an antigen alone, in the absence of an adjuvant, will fail to elicit a humoral or cellular immune response. Adjuvants include, but are not limited to, complete Freund's adjuvant, incomplete Freund's adjuvant, saponin, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols,
25 polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (*bacille Calmette-Guerin*) and *Corynebacterium parvum*. Preferably, the adjuvant is pharmaceutically acceptable.

Various procedures known in the art may be used for the production of polyclonal antibodies for use in the invention. For the production of antibody, various host animals can be immunized by injection with any protein used in the present invention, including but not limited to rabbits, mice, rats, sheep, goats, etc. Various adjuvants
5 may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (*bacille Calmette-Guerin*) and
10 *Corynebacterium parvum*.

For preparation of monoclonal antibodies directed toward the proteins used in the invention any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used. These include but are not limited to the hybridoma technique originally developed by Kohler *et al.*, *Nature*, **256**:495-497
15 (1975), as well as the trioma technique, the human B-cell hybridoma technique [Kozbor *et al.*, *Immunology Today*, **4**:72 (1983)], and the EBV-hybridoma technique to produce human monoclonal antibodies [Cole *et al.*, in *Monoclonal Antibodies and Cancer Therapy*, pp. 77-96, Alan R. Liss, Inc., (1985)]. Immortal, antibody-producing cell lines can be created by techniques other than fusion, such as direct
20 transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. [See, e.g., M. Schreier *et al.*, "Hybridoma Techniques" (1980); Hammerling *et al.*, "Monoclonal Antibodies And T-cell Hybridomas" (1981); Kennett *et al.*, "Monoclonal Antibodies" (1980); see also U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,451,570; 4,466,917; 4,472,500; 4,491,632; and
25 4,493,890].

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, e.g., radioimmunoassay, ELISA (enzyme-linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion

assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), Western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc.

5 In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present
10 invention.

The foregoing antibodies can be used in methods known in the art, *e.g.*, for Western blotting, imaging.

A solid support for use in the present invention will be inert to the reaction conditions for binding. A solid support for use in the present invention may have reactive groups
15 in order to attach a binding partner, such as an antibody to the leptin receptor. In another embodiment, the solid support may be a useful chromatographic support, such as the carbohydrate polymers SEPHAROSE, SEPHADEX, and agarose. As used herein, a solid support is not limited to a specific type of support. Rather, a large number of supports are available and are known to one of ordinary skill in the art.
20 Solid supports include silica gels, resins, derivatized plastic films, glass beads, cotton, plastic beads, alumina gels, magnetic beads, membranes (including but not limited to nitrocellulose, cellulose, nylon, and glass wool filters), plastic and glass dishes or wells, etc. For example, solid supports used for peptide or oligonucleotide synthesis can be used, such as polystyrene resin (*e.g.*, PAM-resin obtained from Bachem Inc.,
25 Peninsula Laboratories, etc.), POLYHIPE® resin (obtained from Aminotech, Canada), polyamide resin (obtained from Peninsula Laboratories), polystyrene resin grafted with polyethylene glycol (TentaGel®, Rapp Polymere, Tubingen, Germany) or polydimethylacrylamide resin (obtained from Milligen/Bioscience, California). The solid support can be formulated as a chromatography support, *e.g.*, in a column; it can

be used in suspension followed by filtration, sedimentation, magnetic association, or centrifugation; or by washing, as in a membrane, well, plastic film, etc.

Phage libraries for Drug Screening.

Phage libraries have been constructed which when infected into host *E. coli* produce random peptide sequences of approximately 10 to 15 amino acids [Parmley and Smith, Gene 73:305-318 (1988), Scott and Smith, Science 249:386-249 (1990)]. Specifically, the phage library can be mixed in low dilutions with permissive *E. coli* in low melting point LB agar which is then poured on top of LB agar plates. After incubating the plates at 37°C for a period of time, small clear plaques in a lawn of *E. coli* will form which represents active phage growth and lysis of the *E. coli*. A representative of these phages can be absorbed to nylon filters by placing dry filters onto the agar plates. The filters can be marked for orientation, removed, and placed in washing solutions to block any remaining absorbent sites. The filters can then be placed in a solution containing, for example, PTP-1D and the leptin receptor containing a phosphorylated tyrosine-985. After a specified incubation period, the filters can be thoroughly washed and developed for autoradiography. Plaques containing the phage that interfere with the PTP-1D leptin receptor binding can then be identified. These phages can be further cloned and then retested. Once the phages have been purified, the binding sequence contained within the phage can be determined by standard DNA sequencing techniques. Once the DNA sequence is known, synthetic peptides can be generated which represents these sequences.

Labels:

Suitable labels include enzymes, fluorophores (e.g., fluoresce isothiocyanate (FITC), phycoerythrin (PE), Texas red (TR), rhodamine, free or chelated lanthanide series salts, especially Eu^{3+} , to name a few fluorophores), chromophores, radioisotopes, chelating agents, dyes, colloidal gold, latex particles, ligands (e.g., biotin), and chemiluminescent agents. When a control marker is employed, the same or different labels may be used for the receptor and control marker.

In the instance where a radioactive label, such as the isotopes ^3H , ^{14}C , ^{32}P , ^{35}S , ^{36}Cl , ^{51}Cr , ^{57}Co , ^{58}Co , ^{59}Fe , ^{90}Y , ^{125}I , ^{131}I , and ^{186}Re are used, known currently available counting procedures may be utilized. In the instance where the label is an enzyme, detection may be accomplished by any of the presently utilized colorimetric, spectrophotometric, fluorospectrophotometric, amperometric or gasometric techniques known in the art.

Direct labels are one example of labels which can be used according to the present invention. A direct label has been defined as an entity, which in its natural state, is readily visible, either to the naked eye, or with the aid of an optical filter and/or applied stimulation, e.g. U.V. light to promote fluorescence. Among examples of colored labels, which can be used according to the present invention, include metallic sol particles, for example, gold sol particles such as those described by Leuvering (U.S. Patent 4,313,734); dye sol particles such as described by Gribnau et al. (U.S. Patent 4,373,932) and May et al. (WO 88/08534); dyed latex such as described by May, *supra*, Snyder (EP-A 0 280 559 and 0 281 327); or dyes encapsulated in liposomes as described by Campbell et al. (U.S. Patent 4,703,017). Other direct labels include a radionucleotide, a fluorescent moiety or a luminescent moiety. In addition to these direct labeling devices, indirect labels comprising enzymes can also be used according to the present invention. Various types of enzyme linked immunoassays are well known in the art, for example, alkaline phosphatase and horseradish peroxidase, lysozyme, glucose-6-phosphate dehydrogenase, lactate dehydrogenase, urease, these and others have been discussed in detail by Eva Engvall in Enzyme Immunoassay ELISA and EMIT in *Methods in Enzymology*, 70. 419-439, 1980 and in U.S. Patent 4,857,453.

Suitable enzymes include, but are not limited to, alkaline phosphatase and horseradish peroxidase.

Other labels for use in the invention include magnetic beads or magnetic resonance imaging labels.

In another embodiment, a phosphorylation site can be created on an antibody of the invention for labeling with ^{32}P , *e.g.*, as described in European Patent No. 0372707 (application No. 89311108.8) by Sidney Pestka, or U.S. Patent No. 5,459,240, issued October 17, 1995 to Foxwell et al.

5 As exemplified herein, proteins, including antibodies, can be labeled by metabolic labeling. Metabolic labeling occurs during *in vitro* incubation of the cells that express the protein in the presence of culture medium supplemented with a metabolic label, such as [^{35}S]-methionine or [^{32}P]-orthophosphate. In addition to metabolic (or biosynthetic) labeling with [^{35}S]-methionine, the invention further contemplates
10 labeling with [^{14}C]-amino acids and [^3H]-amino acids (with the tritium substituted at non-labile positions).

Chimeric and/or fusion proteins containing luciferase or green fluorescent protein [U.S. Patent No. 5625,048 issued April 29, 1997 and WO 97/26333 published July 24, 1997 are hereby incorporated by reference] can also be used. Similarly these
15 proteins can be encoded by reporter genes under the control of a promoter having an activated Stat3 binding site.

Vectors containing the reporter gene as well as nucleic acids encoding the leptin receptor, JAK2, and/or PTP-1D can be introduced into the desired host cells by methods known in the art, *e.g.*, transfection, electroporation, microinjection,
20 transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector transporter (see, *e.g.*, Wu *et al.*, *J. Biol. Chem.*, 267:963-967 (1992); Wu and Wu, *J. Biol. Chem.*, 263:14621-14624 (1988); Hartmut *et al.*, Canadian Patent Application No. 2,012,311, filed March 15, 1990).

25

Drug Assays

Potential drugs can be obtained from any source including the large chemical libraries compiled by large chemical companies such as Merck, Eli Lilly, Hoffman La Roche,

Glaxo Burroughs Welcome, etc. or alternatively can be obtained from phage libraries as detailed above.

The drug assays of the present invention may use any of a large number of protocols known to those having skill in the art. Such assays can measure protein-protein
5 binding and/or stability, protein phosphorylation and/or dephosphorylation and protein activation. For example, protein phosphorylation can be measured by ^{32}P , with antibodies specific for phosphotyrosine as exemplified below or indirectly by the activation of expression of a reporter gene as described above. In this case, the reporter gene is under the control of a promoter having a promoter element which
10 recognizes activated Stat3. Examples of such binding elements include the promoter of acute phase proteins [Zhang *et al.*, *Biochem. Biophys. Res. Comm.*, **237**:90-94 (1997)]; haptoglobin promoter [Kim *et al.*, *J. Biol. Chem.*, **272**:14571-14579 (1997)]; those disclosed by Lamb *et al.* [*Nucleic Acids Res.*, **23**:3283-3289 (1995)] and the SIE, present in the *c-fos* promoter [Ruff-Jamison *et al.*, *J. Biol. Chem.*, **269**:21933-
15 21935 (1994)].

Drug screening assays may be performed in cells that naturally encode the proteins involved in the signal transduction pathway initiated by leptin, preferably a cell is used that is transfected with a plasmid encoding the proteins of interest. For example transient transfections can be performed with 50% confluent U3A cells using the
20 calcium phosphate method as instructed by the manufacturer (Stratagene). Alternatively 293 cells can be used. In certain embodiments the cells can also be modified to contain one or more reporter genes, a heterologous gene encoding a reporter such as luciferase, green fluorescent protein or derivative thereof, chloramphenicol acetyl transferase, β -galactosidase, etc. Such reporter genes can be
25 operable linked to a promoter comprising a Stat3 binding site. Assays for detecting the reporter gene products are readily available in literature for example, luciferase assays can be performed according to the manufacturer's protocol (Promega), and β -galactosidase assays can be performed as described by Ausubel *et al.* [in *Current Protocols in Molecular Biology*, J. Wiley & Sons, Inc. (1994)].

In one example, the transfection reaction can comprise the transfection of a cell with plasmids modified to contain PTP-1D, the leptin receptor, and the JAK2 kinase, such as a pcDNA3 plasmid (Invitrogen). A reporter plasmid that contains a reporter gene under the control of a Stat3 responding promoter can also be included. Although
5 preparation of such plasmids is now routine in the art, many appropriate plasmids are commercially available, e.g., a plasmid with β -galactosidase is available from Stratagene.

Therapeutics

The drugs identified in the present invention have significant therapeutic potential.
10 Preferably, a therapeutically effective amount of such an agent is administered in a pharmaceutically acceptable carrier, diluent, or excipient.

The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similarly untoward reaction, such as gastric upset, dizziness and the like,
15 when administered to a human. In one embodiment, as used herein, the term "pharmaceutically acceptable" may mean approved by a regulatory agency of the federal or a state government or listed in the *U.S. Pharmacopeia* or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the
20 compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are
25 described in Martin, *Remington's Pharmaceutical Sciences*, 18th Ed., Mack Publishing Co., Easton, PA (1990).

The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to reduce by at least about 15%, preferably by at least 50%, more preferably

by at least 90%, and most preferably prevent, a clinically significant deficit in the activity, function and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in the host. Modulation of leptin activity can be useful for reducing body weight (by increasing its activity) or increasing body weight (by decreasing its activity).

Reduction of leptin activity (by developing antagonists, inhibitors, use of neutralizing antibodies, or antisense molecules) should result in weight gain as might be desirable for the treatment of the weight loss associated with cancer, AIDS or anorexia nervosa.

Pharmaceutical Compositions

In another aspect of the present invention, pharmaceutical compositions of the drugs identified are envisioned. Such pharmaceutical compositions may be for administration by injection, or for oral, pulmonary, nasal or other forms of administration. In general, comprehended by the invention are pharmaceutical compositions comprising effective amounts of protein or derivative products of the invention together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions include diluents of various buffer content (*e.g.*, Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (*e.g.*, Tween 80, Polysorbate 80), anti-oxidants (*e.g.*, ascorbic acid, sodium metabisulfite), preservatives (*e.g.*, Thimersol, benzyl alcohol) and bulking substances (*e.g.*, lactose, mannitol); incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc., or into liposomes. Hylauronic acid or other anionic polymers may also be used. Such compositions may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the present proteins and derivatives [See, *e.g.*, Martin, *Remington's Pharmaceutical Sciences*, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712 which is herein incorporated by reference]. The compositions may be prepared in liquid form, or may be in dried powder, such as lyophilized form.

Oral Delivery

Contemplated for use herein are oral solid dosage forms, which are described generally in Martin, *Remington's Pharmaceutical Sciences*, 18th Ed. (1990 Mack Publishing Co. Easton PA 18042) at Chapter 89, which is herein incorporated by reference. Solid dosage forms include tablets, capsules, pills, troches or lozenges, cachets or pellets. Also, liposomal or proteinoid encapsulation may be used to formulate the present compositions (as, for example, proteinoid microspheres reported in U.S. Patent No. 4,925,673). Liposomal encapsulation may be used and the liposomes may be derivatized with various polymers (e.g., U.S. Patent No. 5,013,556). A description of possible solid dosage forms for the therapeutic is given by Marshall, in *Modern Pharmaceutics*, Chapter 10, Banker and Rhodes ed., (1979), herein incorporated by reference.

The present invention may be better understood by reference to the following non-limiting Example, which is provided as exemplary of the invention. The following example is presented in order to more fully illustrate the preferred embodiments of the invention. It should in no way be construed, however, as limiting the broad scope of the invention.

EXAMPLELEPTIN RECEPTOR ACTIVATION OF PTP-1D MODULATESOB-R SIGNAL TRANSDUCTIONSummary:

Binding of leptin to its receptor, Ob-Rb, leads to JAK2 dependent tyrosine phosphorylation of the cytoplasmic domain of receptor and SH2 binding proteins including the STAT3 transcription factor. The phosphotyrosine phosphatase PTP-1D was isolated from bovine and mouse hypothalamus by affinity chromatography using fragments from the Ob-Rb carboxyterminus and shown to be another component of the leptin signal transduction pathway. In vitro binding studies indicated that PTP-1D binds to a phosphotyrosine at position 985 of the Ob-Rb isoform of the leptin

receptor. Co-transfection of Ob-Rb, JAK2 and PTP-1D indicated that the addition of leptin leads to the rapid phosphorylation of PTP-1D. After leptin treatment of cells transfected with wild type receptor the level of JAK2 phosphorylation was decreased in the presence of PTP-1D. The level of JAK2 phosphorylation was not decreased after leptin treatment of cells transfected with PTP-1D and a mutant Y → F 985 leptin receptor. These data suggest that activation of PTP-1D by the leptin receptor results in the dephosphorylates JAK2, and may therefore act to inhibit leptin signal transduction.

Introduction

Leptin is a 16kD hormone that has potent weight reducing effects in vivo. Available data indicate that leptin is the afferent signal in a negative feedback loop regulating food intake and body weight. The leptin receptor is a member of the cytokine family. Leptins anorexigenic effect are dependent on binding to the Ob-Rb isoform of its receptor. The Ob-Rb form of this receptor encodes a long intracytoplasmic domain that includes several motifs for protein-protein interaction. The other forms of this receptor have short cytoplasmic regions and are not capable of initiating signal transduction by themselves. Ob-Rb is highly expressed in the hypothalamus suggesting that this region of the brain is an important site of leptin action. A mutation that specifically ablates Ob-Rb expression in mutant diabetic mice results in obesity and complete leptin resistance. Signal transduction by this class of receptor generally depends on ligand induced phosphorylation of soluble tyrosine receptor kinases such as JAK1, 2, 3, and tyk2. These kinases in turn phosphorylate tyrosine residues on the receptor which serve as docking sites for SH2 proteins. Phosphorylation of SH2 proteins after receptor binding initiates signal transduction.

Results

Leptin binds to a homodimer of the Ob-Rb isoform of its receptor and thereby activates JAK2. While the Stat3 transcription factor is activated by leptin in vivo, the identity of other components of this signal transduction pathway have not yet been identified. To isolate other proteins involved in leptin signaling, Glutathione-S-

Transferase (GST) was fused to three polypeptide fragments spanning each of the three tyrosine residues encoded in the cytoplasmic region of Ob-Rb. The amino acids on the receptor included in each of these three constructs are as indicated (Figure 1A). The three GST Ob-Rb fusion fragments were introduced into bacteria also expressing

5 of the elk tyrosine kinase. The elk kinase led to the specific tyrosine phosphorylation of the Ob-Rb fragments. Treatment of the fusion fragments with thrombin followed by immunoblotting with an anti phosphotyrosine antibody confirmed that only the Ob-Rb peptide sequence was phosphorylated. Each of the three fusion fragments were incubated with protein extracts from bovine and mouse hypothalamus. The bound

10 proteins were precipitated, eluted and fractionated by SDS PAGE. A single protein band of 64kD was observed after Coomassie Blue staining of protein precipitated after incubation with the fusion protein spanning the first tyrosine at position 985 (Figure 1B). Precipitation of the 64kD protein was dependent on the presence of a phosphotyrosine at position 985 as the fusion fragment without a phosphotyrosine

15 was not active. The 64 kD protein was sequenced and the sequence was found to match PTP-1D. PTP-1D is a phosphotyrosine phosphatase. The relative efficiency of binding of PTP-1D to the GST Ob-Rb fragment 1 (GST-ObRbTyr1) was compared to that of Stat3 for the GST fusion protein spanning fragment 3 (GST-ObRbTyr3). Stat3 is known to be activated by Ob-Rb in vivo and a Stat3 binding motif, YXXL, is found

20 in the region of tyrosine 1138. Ob-Rb fragments 1 and 3 were incubated with hypothalamic extracts and immunoblotted using anti-Stat3 and anti PTP-1D specific antisera. PTP-1D immunoactivity was found specifically in the precipitate using the GST Ob-Rb fusion fragment 1 (Figure 2A) while Stat3 immunoactivity was found only in the material precipitated after incubation with GST Ob-Rb fragment 3 (Figure

25 2B). The intensity of the PTP-1D signal was much greater than that of Stat3.

These data suggested that PTP-1D might play a role in leptin signal transduction. cDNA clones encoding Ob-Rb, JAK2, PTP-1D, and Ob-Ra were transfected in various combinations into 293 cells (Figure 3). Ob-Ra is a short form of the leptin receptor that does not encode any tyrosine residues in its 34 amino acid

30 carboxyterminus. The transfected cells were treated with either PBS or leptin. In

each case the cellular proteins were immunoprecipitated using a specific anti-PTP-1D antibody and detected using an anti-phosphotyrosine antibody. In separate experiments a total cell lysate was immunoblotted using the anti-phosphotyrosine antibody. Phosphorylation of PTP-1D was observed only in the cells that were co-
5 transfected with Ob-R and JAK2 (Figure 3). In the untreated Ob-Rb transfected cells, there was a low level of phosphorylation of PTP-1D that increased six fold when leptin was added to the culture. Curiously the basal level of phosphorylation was higher in the cells that were transfected with Ob-Ra but PTP-1D phosphorylation was not increased by the addition of leptin. Thus leptin binding to Ob-Rb leads to the
10 phosphorylation of PTP-1D. The basis for the leptin independent activation of PTP-1D in the Ob-Ra transfected cells is unknown.

The time course of PTP-1D activation by leptin in Ob-Rb transfected cells was compared between wild type and a Y→F 985 mutant receptor (Figure 4). Leptin induced PTP-1D phosphorylation in the cells transfected with the wild type receptor
15 within 5 minutes and PTP-1D remained phosphorylated after one hour. The level of phosphorylation was substantially reduced in the cells transfected with the mutant receptor at all time points.

The possible function of PTP-1D in signal transduction was further assessed by following the time course and level of phosphorylation of the various components of
20 the leptin signal transduction pathway. Cells were transfected with either the wild type or Y→F 985 mutant receptor in the presence or absence of PTP-1D (Figure 5). Without PTP-1D, JAK2 was highly phosphorylated in response to leptin after 30 and 60 minutes of treatment. In this case, the level of phosphorylation of the wild type receptor was greater than that of the Y→F 985 mutant receptor. When PTP-1D was
25 added, this relationship was reversed and the level of JAK2 phosphorylation was much greater in the cells that were transfected with the mutant form of the receptor. These data suggest activation of PTP-1D after binding to Phosphotyrosine 985 leads to dephosphorylation of JAK2. The present data indicate that PTP-1D is a component of the leptin signal transduction pathway. The data further indicate that when

activated, PTP-1D can dephosphorylate JAK2 and thereby inhibit the leptin signal transduction pathway. This role is analogous to that played by PTP-1C, another phosphotyrosine phosphatase, which dephosphorylates JAK2 in hematopoietic cells. Previous studies have suggested that PTP-1D acts as an activator of signal
5 transduction. Thus corkscrew, the *Drosophila* homologue of PTP-1D, is required for signal transduction by the sevenless (DOS) receptor in R7 photoreceptor cells. In R7 cells corkscrew has been shown to lead to the dephosphorylation of daughter of sevenless leading to R7 differentiation. PTP-1D has also been suggested to play a positive role in signal transduction pathways activated by prolaction, IL3, PDGF and
10 other peptide hormones. The data presented here indicate that in the setting of leptin signal transduction, PTP-1D acts as an inhibitor of signal transduction by decreasing the level of JAK2 phosphorylation. PTP-1D has also been suggested to reduce signal transduction in T cells that are inhibited by CTL-4.

Therefore, the inhibition of PTP-1D could enhance leptin action. However, PTP-1D
15 function is not likely to be limited to leptin action as it is expressed ubiquitously. In addition, mice with induced mutations in PTP-1D are not viable and die after about 10.5 days of development. Finally, PTP-1D has also been shown to associate with the prolaction PDGF, IL3, IL4 and receptors. Nevertheless, PTP-1D appears to modulate leptin action in vivo.

20 The present invention is not be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

25 It is further understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for nucleic acids or polypeptides are approximate, and are provided for description.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

- 1 1. A method of identifying a modulator of the binding of a phosphorylated leptin
2 receptor with PTP-1D comprising:
3 (a) contacting the phosphorylated leptin receptor or phosphorylated
4 fragment thereof with PTP-1D or fragment thereof in the presence and absence of an
5 agent under conditions in which in the absence of the agent the binding of the
6 phosphorylated leptin receptor or phosphorylated fragment with PTP-1D or fragment
7 thereof can be detected; and
8 (b) detecting the binding of the phosphorylated leptin receptor or
9 phosphorylated fragment thereof with PTP-1D or fragment thereof; wherein when an
10 increase in binding is detected in the presence of the agent, the agent is identified as a
11 modulator that enhances the binding of the phosphorylated leptin receptor or
12 phosphorylated fragment thereof with PTP-1D or fragment thereof, and when a
13 decrease in binding is detected in the presence of the agent, the agent is identified as a
14 modulator that inhibits the binding of the phosphorylated leptin receptor or
15 phosphorylated fragment thereof with PTP-1D or fragment thereof; wherein the
16 phosphorylated leptin receptor or phosphorylated fragment thereof contains a
17 phosphorylated tyrosine-985.
- 1 2. The method of Claim 1 wherein the phosphorylated leptin receptor or
2 phosphorylated fragment thereof is bound to a solid support.
- 1 3. The method of Claim 2 wherein PTP-1D or fragment thereof is labeled.
- 1 4. The method of Claim 1 wherein the phosphorylated fragment is part of a
2 fusion protein.
- 1 5. The method of Claim 4 wherein the fusion protein comprises the
2 phosphorylated fragment fused to a protein selected from the group consisting of
3 glutathione-S-transferase, and green fluorescent protein.

1 6. A method of identifying a modulator of the phosphorylated leptin
2 receptor-dependent PTP-1D phosphorylation comprising:

3 (a) contacting a phosphorylated leptin receptor or phosphorylated fragment
4 thereof with PTP-1D and JAK2 kinase in the presence and absence of an agent under
5 conditions in which in the absence of the agent the phosphorylated leptin receptor or
6 phosphorylated fragment thereof stimulates the phosphorylation of PTP-1D; wherein
7 the phosphorylated leptin receptor or phosphorylated fragment thereof contains a
8 phosphorylated tyrosine-985;

9 (b) determining the amount of phosphorylation of PTP-1D; wherein when
10 either an increase or decrease in the phosphorylation is determined in the presence of
11 the agent relative to in the absence of the agent, the agent is identified as a potential
12 modulator of the leptin receptor-dependent phosphorylation of PTP-1D; and

13 (c) contacting the potential modulator with PTP-1D under the conditions
14 of step (a) except in the absence of the phosphorylated leptin receptor or
15 phosphorylated fragment that contains a phosphorylated tyrosine-985; wherein when
16 no significant change in phosphorylation is determined in the presence of the potential
17 modulator relative to in the absence of the potential modulator, the potential
18 modulator is identified as a modulator of the leptin-dependent phosphorylation of
19 PTP-1D.

1 7. The method of Claim 6 wherein the phosphorylated fragment is part of a
2 fusion protein.

1 8. The method of Claim 7 wherein the fusion protein comprises the
2 phosphorylated fragment fused to a protein selected from the group consisting of
3 glutathione-S-transferase, and green fluorescent protein.

1 9. A method of identifying a potential modulator and/or modulator of the
2 leptin-dependent PTP-1D phosphorylation *in situ* comprising:

- 3 (a) contacting a cell with leptin in the presence and absence of an agent
4 under conditions in which in the absence of the agent leptin induces the
5 phosphorylation of PTP-1D; wherein the cell comprises PTP-1D, JAK2, and a leptin
6 receptor which contains tyrosine-985; and
7 (b) determining the amount of phosphorylation of PTP-1D; wherein when
8 an increase or decrease in phosphorylation of PTP-1D is determined in the presence of
9 the agent relative to in the absence of the agent, the agent is identified as a potential
10 modulator of the leptin-dependent phosphorylation of PTP-1D.

1 10. The method of Claim 9 wherein the cell is transfected with vectors which
2 encode PTP-1D, JAK2, and a leptin receptor which contains tyrosine-985.

1 11. The method of Claim 9 further comprising:

- 2 (c) contacting a second cell with leptin and the potential modulator under
3 the conditions of those of step (a) except wherein leptin cannot induce the
4 phosphorylation of PTP-1D; and wherein the second cell comprises PTP-1D, JAK2,
5 and a leptin receptor that does not contain tyrosine-985; and
6 (d) determining the amount of phosphorylation of PTP-1D; wherein when
7 no significant change in phosphorylation is determined in the presence of the potential
8 modulator relative to in the absence of the potential modulator, the potential
9 modulator is identified as modulator of the leptin-dependent phosphorylation of
10 PTP-1D.

1 12. The method of Claim 11 wherein the leptin receptor that does not contain
2 tyrosine-985 is selected from the group consisting of Ob-Ra and Ob-Rb containing a
3 phenylalanine-985.

1 13. The method of Claim 11 wherein the second cell is transfected with vectors
2 which encode PTP-1D, JAK2, and the leptin receptor that does not contain
3 tyrosine-985.

1 14. The method of Claim 11 wherein the modulator enhances the leptin
2 receptor-dependent phosphorylation of PTP-1D.

1 15. The method of Claim 11 wherein the modulator inhibits the leptin
2 receptor-dependent phosphorylation of PTP-1D.

1 16. A method of identifying a drug useful in a weight loss diet regimen
2 comprising:

3 (a) administering multiple doses of the modulator of Claim 15 to a test
4 animal and multiple doses of a placebo to a control animal over a prescribed time
5 period; and

6 (b) determining the change in weight of the test animal and the control
7 animal during the prescribed time period; wherein a modulator that causes the test
8 animal to lose weight relative to the control animal is selected as a drug that is useful
9 in a weight loss diet regimen.

1 17. A method of identifying a modulator of the leptin-dependent PTP-1D
2 dephosphorylation of the JAK2 kinase *in situ* comprising:

3 (a) contacting a cell with leptin in the presence and absence of an agent
4 under conditions in which in the absence of the agent, leptin induces the PTP-1D
5 dephosphorylation of JAK2; wherein the cell comprises PTP-1D, JAK2, and a leptin
6 receptor which contains tyrosine-985;

7 (b) determining the amount of phosphorylation of JAK2; wherein when a
8 decrease or increase in phosphorylation of JAK2 is determined in the presence of the
9 agent relative to in the absence of the agent, the agent is identified as a potential
10 modulator of the leptin-dependent PTP-1D dephosphorylation of JAK2; and

11 (c) contacting the cell with the potential modulator under the conditions of
12 step (a) but in the absence of leptin; wherein when no significant change in
13 phosphorylation is determined in the presence of the potential modulator relative to in
14 the absence of the potential modulator, the potential modulator is identified as a
15 modulator of the leptin-dependent PTP-1D dephosphorylation of JAK2.

1 18. A method of identifying an inhibitor of the leptin-dependent PTP-1D

2 phosphorylation *in situ* comprising:

3 (a) contacting a first cell with leptin in the presence and absence of an agent
4 under conditions in which in the absence of the agent leptin induces the expression of
5 a reporter gene operably under the control of a promoter containing a binding site for
6 activated Stat3;

7 (b) measuring the level of expression of the reporter gene contained by the
8 first cell; wherein the binding of activated Stat3 protein to the binding site induces the
9 expression of the reporter gene; wherein the cell comprises PTP-1D, JAK2, Stat3 and
10 a leptin receptor which contains tyrosine-985; and wherein when the presence of the
11 agent results in an increase in the level of expression of the reporter gene relative to in
12 the absence of the agent, the agent is identified as a candidate inhibitor of the
13 leptin-dependent PTP-1D phosphorylation;

14 (c) contacting a second cell with leptin in the presence and absence of the
15 candidate inhibitor under conditions in which in the absence of the candidate inhibitor
16 leptin induces the phosphorylation of PTP-1D; wherein the second cell comprises
17 PTP-1D, JAK2, and a leptin receptor which contains tyrosine-985;

18 (d) determining the amount of phosphorylation of PTP-1D of step (c);
19 wherein when a decrease in phosphorylation of PTP-1D is determined in the presence
20 of the candidate inhibitor relative to in the absence of the candidate inhibitor, the
21 candidate inhibitor is identified as a potential inhibitor of the leptin-dependent
22 phosphorylation of PTP-1D;

23 (e) contacting a third cell with leptin and the inhibitor under the conditions
24 of those of step (c) except wherein leptin cannot induce the phosphorylation of PTP-
25 1D; and wherein the third cell comprises PTP-1D, JAK2, and a leptin receptor that
26 does not contain tyrosine-985; and

27 (f) determining the amount of phosphorylation of PTP-1D of step (e);
28 wherein when no decrease in phosphorylation is determined in the presence of the
29 potential inhibitor relative to in the absence of the potential inhibitor, the potential
30 inhibitor is identified as an inhibitor of leptin-dependent phosphorylation of PTP-1D.

1 19. The method of Claim 18 wherein the reporter gene encodes a protein selected
2 from the group consisting of luciferase and green fluorescent protein.

1 20. A method of identifying a drug useful in a weight loss diet regimen
2 comprising:

3 (a) administering multiple doses of the inhibitor of Claim 18 to a test
4 animal and multiple doses of a placebo to a control animal over a prescribed time
5 period; and

6 (b) determining the change in weight of the test animal and the control
7 animal during the prescribed time period; wherein an inhibitor that causes the test
8 animal to lose weight relative to the control animal is selected as a drug that is useful
9 in a weight loss diet regimen.

SEQUENCE LISTING

<110> Li, Cai

Friedman, Jeffrey M.

<120> METHODS OF IDENTIFYING AGENTS THAT MODULATE LEPTIN
ACTIVITY

<130> 600-1-205 PCT

<140>

<141>

<160> 8

<170> PatentIn Ver. 2.0

<210> 1

<211> 3489

<212> DNA

<213> Mus musculus

<400> 1

atgatgtgtc agaaattcta tgtgggttttg ttacactggg aattttcttta tgtgatagct 60
gcacttaacc tggcatatcc aatctctccc tggaaattta agttgttttg tggaccaccg 120
aacacaaccg atgactcctt tctctcacct gctggagccc caaacaatgc ctgggctttg 180
aagggggctt ctgaagcaat tgttgaagct aaatttaatt caagtgggtat ctacgttcct 240
gagttatcca aaacagtctt cactgtttgc tttgggaatg agcaagggtca aaactgctct 300
gcactcacag acaacactga agggaagaca ctggcttcag tagtgaaggc ttcagttttt 360
cgccagctag gtgtaaactg ggacatagag tgctggatga aaggggactt gacattattc 420
atctgtcata tggagccatt acctaagaac cccttcaaga attatgactc taagggtccat 480

cttttatatg atctgcctga agtcatagat gattcgccctc tgccccact gaaagacagc 540
tttcagactg tccaatgcaa ctgcagtctt cggggatgtg aatgtcatgt gccggtaccc 600
agagccaaac tcaactacgc tcttctgatg tattttgaaa tcacatctgc cgggtgtgagt 660
tttcagtcac ctctgatgtc actgcagccc atgcttggtg tgaaaccga tccaccctta 720
ggtttgcata tggaagtcac agatgatggg aatttaaaga tttcttgga cagccaaaca 780
atggcaccat ttccgcttca atatcagggtg aaatathtag agaattctac aattgtaaga 840
gaggctgctg aaattgtctc agctacatct ctgctggtag acagtgtgct tcctggatct 900
tcatatgagg tccagggtgag gagcaagaga ctggatgggt caggagtctg gagtgactgg 960
agttcacctc aagtctttac cacacaagat gttgtgtatt ttccaccaa aattctgact 1020
agtgttggt cgaatgcttc ttttcattgc atctacaaaa acgaaaacca gattatctcc 1080
tcaaaacaga tagtttggtg gaggaatcta gctgagaaaa tccctgagat acagtacagc 1140
attgtgagtg accgagttag caaagttacc ttctccaacc tgaaagccac cagacctga 1200
gggaagttta cctatgacgc agtgactgc tgcaatgagc aggcgtgcca tcaccgctat 1260
gtgaattat acgtgatcga tgtcaatata aatatatcat gtgaaactga cgggtactta 1320
actaaaatga cttgcagatg gtcaccacgc acaatccaat cactagtggg aagcactgtg 1380
cagctgaggt atcacaggcg cagcctgtat tgcctgata gtccatctat tcatcctacg 1440
tctgagccca aaaactgcgt cttacagaga gacggctttt atgaatgtgt tttccagcca 1500
atctttctat tatctggcta tacaatgtgg atcaggatca accattcttt aggttcactt 1560
gactcgccac caacgtgtgt ccttctgac tccgtagtaa aaccactacc tccatctaac 1620
gtaaaagcag agattactgt aaacactgga ttattgaaag tatcttgga aaagccagtc 1680
tttccggaga ataaccttca attccagatt cgatatggct taagtggaaa agaaatacaa 1740
tggaagacac atgaggtatt cgatgcaaag tcaaagtctg ccagcctgct ggtgtcagac 1800
ctctgtgcag tctatgtggt ccagggttcgc tgccggcggt tggatggact aggatattgg 1860
agtaattgga gcagtccagc ctatacgctt gtcattggtg taaaagtctc tatgagaggg 1920
cctgaatttt ggagaaaaat ggatggggac gttactaaaa aggagagaaa tgtcaccttg 1980
ctttggaagc ccctgacgaa aaatgactca ctgtgtagtg tgaggaggta cgtgggtgaag 2040
catcgtactg cccacaatgg gacgtgggtc gaagatgtgg gaaatcggac caatctcact 2100
ttcctgtgga cagaaccagc gcacactgtt acagttctgg ctgtcaattc cctcggcgct 2160
tccctgtgga attttaacct taccttctca tggcccatga gtaaagtga tgctgtggag 2220
tactcagtg cttatcccct gagcagcagc tgtgtcatcc tttctggac actgtcacct 2280
gatgattata gtctgttata tctggttatt gaatggaaga tccttaatga agatgatgga 2340

atgaagtggc ttagaattcc ctgcaatggt aaaaagtttt atatccacga taatttttatt 2400
 cccatcgaga aatatcagtt tagtctttac ccagtattta tggaaggagt tggaaaacca 2460
 aagataatta atggttttcac caaagatgct atcgacaagc agcagaatga cgcagggctg 2520
 tatgtcattg taccataat tatttcctct tgtgtcctac tgctcggaac actgttaatt 2580
 tcacaccaga gaatgaaaaa gttgttttgg gacgatgttc caaaccccaa gaattgttcc 2640
 tgggcacaag gactgaattt ccaaaagcct gaaacatttg agcatctttt taccaagcat 2700
 gcagaatcag tgatatttgg tcctcttctt ctggagcctg aaccatttc agaagaaatc 2760
 agtgtcgata cagcttgga aaataaagat gagatgggtc cagcagctat ggtctccctt 2820
 cttttgacca caccagacc tgaaagcagt tctatttgta ttagtgacca gtgtaacagt 2880
 gctaacttct ctgggtctca gagcaccag gtaacctgtg aggatgagtg tcagagacaa 2940
 ccctcagtta aatatgcaac tctggtcagc aacgataaac tagtggaac tgatgaagag 3000
 caagggttta tccatagtcc tgtcagcaac tgcattcca gtaatcattc ccactgagg 3060
 cagtcttct ctagcagctc ctgggagaca gaggccaga catttttctt tttatcagac 3120
 cagcaacca ccatgatttc accacaactt tcattctcgg ggttgatga gcttttgga 3180
 ctggaggga gttttctga agaaaatcac agggagaagt ctgtctgtta tctaggagtc 3240
 acctccgtca acagaagaga gagtgggtgtg cttttgactg gtgaggcagg aatcctgtgc 3300
 acattcccag ccagtggtt gttcagtgc atcaggatcc tccaggagag atgctcacac 3360
 tttgtagaaa ataatttgag tttagggacc tctggtgaga actttgtacc ttacatgcc 3420
 caatttcaaa cctgttcac gcacagtcac aagataatgg agaataagat gtgtgactta 3480
 actgtgtaa 3489

<210> 2

<211> 1162

<212> PRT

<213> Mus musculus

<400> 2

Met Met Cys Gln Lys Phe Tyr Val Val Leu Leu His Trp Glu Phe Leu

1

5

10

15

Tyr Val Ile Ala Ala Leu Asn Leu Ala Tyr Pro Ile Ser Pro Trp Lys

20 25 30

Phe Lys Leu Phe Cys Gly Pro Pro Asn Thr Thr Asp Asp Ser Phe Leu
35 40 45

Ser Pro Ala Gly Ala Pro Asn Asn Ala Ser Ala Leu Lys Gly Ala Ser
50 55 60

Glu Ala Ile Val Glu Ala Lys Phe Asn Ser Ser Gly Ile Tyr Val Pro
65 70 75 80

Glu Leu Ser Lys Thr Val Phe His Cys Cys Phe Gly Asn Glu Gln Gly
85 90 95

Gln Asn Cys Ser Ala Leu Thr Asp Asn Thr Glu Gly Lys Thr Leu Ala
100 105 110

Ser Val Val Lys Ala Ser Val Phe Arg Gln Leu Gly Val Asn Trp Asp
115 120 125

Ile Glu Cys Trp Met Lys Gly Asp Leu Thr Leu Phe Ile Cys His Met
130 135 140

Glu Pro Leu Pro Lys Asn Pro Phe Lys Asn Tyr Asp Ser Lys Val His
145 150 155 160

Leu Leu Tyr Asp Leu Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro
165 170 175

Leu Lys Asp Ser Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly
180 185 190

Cys Glu Cys His Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu
195 200 205

Leu Met Tyr Leu Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro
210 215 220

Leu Met Ser Leu Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu
225 230 235 240

Gly Leu His Met Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser Trp
245 250 255

Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr
260 265 270

Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala Glu Ile Val Ser Ala
275 280 285

Thr Ser Leu Leu Val Asp Ser Val Leu Pro Gly Ser Ser Tyr Glu Val
290 295 300

Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly Val Trp Ser Asp Trp
305 310 315 320

Ser Ser Pro Gln Val Phe Thr Thr Gln Asp Val Val Tyr Phe Pro Pro
325 330 335

Lys Ile Leu Thr Ser Val Gly Ser Asn Ala Ser Phe His Cys Ile Tyr
340 345 350

Lys Asn Glu Asn Gln Ile Ile Ser Ser Lys Gln Ile Val Trp Trp Arg

355

360

365

Asn Leu Ala Glu Lys Ile Pro Glu Ile Gln Tyr Ser Ile Val Ser Asp

370

375

380

Arg Val Ser Lys Val Thr Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg

385

390

395

400

Gly Lys Phe Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys

405

410

415

His His Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile

420

425

430

Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser

435

440

445

Pro Ser Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr

450

455

460

His Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr

465

470

475

480

Ser Glu Pro Lys Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr Glu Cys

485

490

495

Val Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp Ile Arg

500

505

510

Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr Cys Val Leu

515	520	525
Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Asn Val Lys Ala Glu		
530	535	540
Ile Thr Val Asn Thr Gly Leu Leu Lys Val Ser Trp Glu Lys Pro Val		
545	550	555
		560
Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg Tyr Gly Leu Ser Gly		
	565	570
		575
Lys Glu Ile Gln Trp Lys Thr His Glu Val Phe Asp Ala Lys Ser Lys		
	580	585
		590
Ser Ala Ser Leu Leu Val Ser Asp Leu Cys Ala Val Tyr Val Val Gln		
	595	600
		605
Val Arg Cys Arg Arg Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser		
610	615	620
Ser Pro Ala Tyr Thr Leu Val Met Asp Val Lys Val Pro Met Arg Gly		
625	630	635
		640
Pro Glu Phe Trp Arg Lys Met Asp Gly Asp Val Thr Lys Lys Glu Arg		
	645	650
		655
Asn Val Thr Leu Leu Trp Lys Pro Leu Thr Lys Asn Asp Ser Leu Cys		
	660	665
		670
Ser Val Arg Arg Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr		
675	680	685

Trp Ser Glu Asp Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr
690 695 700

Glu Pro Ala His Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala
705 710 715 720

Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys Val
725 730 735

Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser Cys Val
740 745 750

Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu Leu Tyr Leu
755 760 765

Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly Met Lys Trp Leu
770 775 780

Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile His Asp Asn Phe Ile
785 790 795 800

Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr Pro Val Phe Met Glu Gly
805 810 815

Val Gly Lys Pro Lys Ile Ile Asn Gly Phe Thr Lys Asp Ala Ile Asp
820 825 830

Lys Gln Gln Asn Asp Ala Gly Leu Tyr Val Ile Val Pro Ile Ile Ile
835 840 845

Ser Ser Cys Val Leu Leu Leu Gly Thr Leu Leu Ile Ser His Gln Arg

850

855

860

Met Lys Lys Leu Phe Trp Asp Asp Val Pro Asn Pro Lys Asn Cys Ser

865

870

875

880

Trp Ala Gln Gly Leu Asn Phe Gln Lys Pro Glu Thr Phe Glu His Leu

885

890

895

Phe Thr Lys His Ala Glu Ser Val Ile Phe Gly Pro Leu Leu Leu Glu

900

905

910

Pro Glu Pro Ile Ser Glu Glu Ile Ser Val Asp Thr Ala Trp Lys Asn

915

920

925

Lys Asp Glu Met Val Pro Ala Ala Met Val Ser Leu Leu Leu Thr Thr

930

935

940

Pro Asp Pro Glu Ser Ser Ser Ile Cys Ile Ser Asp Gln Cys Asn Ser

945

950

955

960

Ala Asn Phe Ser Gly Ser Gln Ser Thr Gln Val Thr Cys Glu Asp Glu

965

970

975

Cys Gln Arg Gln Pro Ser Val Lys Tyr Ala Thr Leu Val Ser Asn Asp

980

985

990

Lys Leu Val Glu Thr Asp Glu Glu Gln Gly Phe Ile His Ser Pro Val

995

1000

1005

Ser Asn Cys Ile Ser Ser Asn His Ser Pro Leu Arg Gln Ser Phe Ser

1010 1015 1020
Ser Ser Ser Trp Glu Thr Glu Ala Gln Thr Phe Phe Leu Leu Ser Asp
1025 1030 1035 1040
Gln Gln Pro Thr Met Ile Ser Pro Gln Leu Ser Phe Ser Gly Leu Asp
1045 1050 1055
Glu Leu Leu Glu Leu Glu Gly Ser Phe Pro Glu Glu Asn His Arg Glu
1060 1065 1070
Lys Ser Val Cys Tyr Leu Gly Val Thr Ser Val Asn Arg Arg Glu Ser
1075 1080 1085
Gly Val Leu Leu Thr Gly Glu Ala Gly Ile Leu Cys Thr Phe Pro Ala
1090 1095 1100
Gln Cys Leu Phe Ser Asp Ile Arg Ile Leu Gln Glu Arg Cys Ser His
1105 1110 1115 1120
Phe Val Glu Asn Asn Leu Ser Leu Gly Thr Ser Gly Glu Asn Phe Val
1125 1130 1135
Pro Tyr Met Pro Gln Phe Gln Thr Cys Ser Thr His Ser His Lys Ile
1140 1145 1150
Met Glu Asn Lys Met Cys Asp Leu Thr Val
1155 1160

<211> 3800

<212> DNA

<213> Homo sapiens

<400> 3

ggcagcagcc ggtctggctt gggcaggctg cccgggccgt ggcaggaagc cggaagcagc 60
cgcgccccc gttcgggaga catggcgggc gttaaagctc tcgtggcatt atccttcagt 120
ggggctattg gactgacttt tcttatgctg ggatgtgcct tagaggatta tgggtgtact 180
tctctgaagt aagatgattt gtcaaaaatt ctgtgtgggt ttgttacatt gggaatttat 240
ttatgtgata actgcgttta acttgtcata tccaattact ccttggagat ttaagttgtc 300
ttgcatgcc ccaaattcaa cctatgacta cttccttttg cctgctggac tctcaaagaa 360
tacttcaa at tcgaatggac attatgagac agctgttgaa cctaagttta attcaagtgg 420
tactcacttt tctaacttat ccaaaacaac tttccactgt tgctttcgga gtgagcaaga 480
tagaaactgc tccttatgtg cagacaacat tgaaggaaag acatttggtt caacagtaaa 540
ttcttttagtt tttcaacaaa tagatgcaaa ctggaacata cagtgtctggc taaaaggaga 600
cttaaaatta ttcattctgtt atgtggagtc attatttaag aatctattca ggaattataa 660
ctataaggtc catcttttat atgttctgcc tgaagtgtta gaagattcac ctctgggtcc 720
ccaaaaaggc agttttcaga tggttcactg caattgcagt gttcatgaat gttgtgaatg 780
tcttgtgcct gtgccaacag ccaaactcaa cgacactctc cttatgtgtt tgaaaatcac 840
atctgggtga gtaattttcc agtcacctct aatgtcagtt cagcccataa atatgggtgaa 900
gcctgatcca ccattagggt tgcatatgga aatcacagat gatggtaatt taaagatttc 960
ttgggtccagc ccaccattgg taccatttcc acttcaatat caagtgaaat attcagagaa 1020
ttctacaaca gttatcagag aagctgacaa gattgtctca gctacatccc tgctagtaga 1080
cagtatactt cctgggtctt cgtatgaggt tcagggtgagg ggcaagagac tggatggccc 1140
aggaatctgg agtgactgga gtactcctcg tgtctttacc acacaagatg tcatatactt 1200
tccacctaaa attctgacaa gtgttggtgc taatgtttct tttcactgca tctataagaa 1260
ggaaaacaag attgttcctt caaaagagat tgtttggtgg atgaatttag ctgagaaaat 1320
tcctcaaagc cagtatgatg ttgtgagtga tcatgttagc aaagttactt ttttcaatct 1380
gaatgaaacc aaacctcgag gaaagtttac ctatgatgca gtgtactgct gcaatgaaca 1440
tgaatgccat catcgctatg ctgaattata tgtgattgat gtcaatatca atatctcatg 1500
tgaaactgat ggtacttaa ctaaaatgac ttgcagatgg tcaaccagta caatccagtc 1560

acttgcgga agcactttgc aattgaggta tcataggagc agcctttact gttctgatat 1620
tccatctatt catcccatat ctgagcccaa agattgctat ttgcagagtg atgggttttta 1680
tgaatgcatt ttccagccaa tcttcttatt atctggctac acaatgtgga ttaggatcaa 1740
tcactctcta ggttcacttg actctccacc aacatgtgtc cttcctgatt ctgtggtgaa 1800
gccactgcct ccatccagtg tgaaagcaga aattactata aacattggat tattgaaaat 1860
atcttgggaa aagccagtct ttccagagaa taaccttcaa ttccagattc gctatgggtt 1920
aagtggaaaa gaagtacaat ggaagatgta tgagggttat gatgcaaat caaaatctgt 1980
cagtctccca gttccagact tgtgtgcagt ctatgctgtt cagggtgcgt gtaagaggct 2040
agatggactg ggatattgga gtaattggag caatccagcc tacacagttg tcatggatat 2100
aaaagttcct atgagaggac ctgaattttg gagaataatt aatggagata ctatgaaaaa 2160
ggagaaaaat gtcactttac tttggaagcc cctgatgaaa aatgactcat tgtgcagtgt 2220
tcagagatat gtgataaacc atcatacttc ctgcaatgga acatggtcag aagatgtggg 2280
aatcacacg aaattcactt tcctgtggac agagcaagca catactgtta cggttctggc 2340
catcaattca attggtgctt ctgttgcaaa ttttaattta accttttcat ggcctatgag 2400
caaagtaaat atcgtgcagt cactcagtgc ttatccttta aacagcagtt gtgtgattgt 2460
ttcctggata ctatcaccca gtgattacaa gctaattgat tttattattg agtggaaaaa 2520
tcttaatgaa gatggtgaaa taaaatggct tagaatctct tcatctgtta agaagtatta 2580
tatccatgat cttttatcc ccattgagaa gtaccagttc agtctttacc caatatttat 2640
ggaaggagtg ggaaaaccaa agataattaa tagtttctact caagatgata ttgaaaaaca 2700
ccagagtgat gcagggttat atgtaattgt gccagtaatt atttcctctt ccatcttatt 2760
gcttggaaaca ttattaatat cacaccaaag aatgaaaaag ctattttggg aagatgttcc 2820
gaaccccaag aattgttctt gggcacaaag acttaatttt cagaagccag aaacgtttga 2880
gcatcttttt atcaagcata cagcatcagt gacatgtggt cctcttcttt tggagcctga 2940
aacaatttca gaagatatca gtgttgatac atcatggaaa aataaagatg agatgatgcc 3000
aacaactgtg gtctctctac tttcaacaac agatcttgaa aagggttctg tttgtattag 3060
tgaccagttc aacagtgtta acttctctga ggctgagggt actgaggtaa cctatgaggc 3120
cgaaagccag agacaaccct ttgttaaata cgccacgctg atcagcaact ctaaaccaag 3180
tgaaactggt gaagaacaag ggcttataaa tagttcagtc accaagtgtt tctctagcaa 3240
aaattctccg ttgaaggatt ctttctctaa tagctcatgg gagatagagg ccaggcatt 3300
ttttatatta tcagatcagc atcccaacat aatttcacca cacctcacat tctcagaagg 3360
attggatgaa cttttgaaat tggagggaaa tttccctgaa gaaaataatg ataaaaagtc 3420

tatctattat ttaggggtca cctcaatcaa aaagagagag agtgggtgtgc ttttgactga 3480
caagtcaagg gtatcgtgcc cattcccagc cccctgttta ttcacggaca tcagagttct 3540
ccaggacagt tgctcacact ttgtagaaaa taatatcaac ttaggaactt ctagtaagaa 3600
gacttttgca tcttacatgc ctcaattcca aacttgttct actcagactc ataagatcat 3660
ggaaaacaag atgtgtgacc taactgtgta atttcactga agaaaccttc agatttgtgt 3720
tataatgggt aatataaagt gtaatagatt atagttgtgg gtgggagaga gaaaagaaac 3780
cagagtccaa atttgaaaat 3800

<210> 4

<211> 1165

<212> PRT

<213> Homo sapiens

<400> 4

Met Ile Cys Gln Lys Phe Cys Val Val Leu Leu His Trp Glu Phe Ile

1 5 10 15

Tyr Val Ile Thr Ala Phe Asn Leu Ser Tyr Pro Ile Thr Pro Trp Arg

20 25 30

Phe Lys Leu Ser Cys Met Pro Pro Asn Ser Thr Tyr Asp Tyr Phe Leu

35 40 45

Leu Pro Ala Gly Leu Ser Lys Asn Thr Ser Asn Ser Asn Gly His Tyr

50 55 60

Glu Thr Ala Val Glu Pro Lys Phe Asn Ser Ser Gly Thr His Phe Ser

65 70 75 80

Asn Leu Ser Lys Thr Thr Phe His Cys Cys Phe Arg Ser Glu Gln Asp

85 90 95

Arg Asn Cys Ser Leu Cys Ala Asp Asn Ile Glu Gly Lys Thr Phe Val

100

105

110

Ser Thr Val Asn Ser Leu Val Phe Gln Gln Ile Asp Ala Asn Trp Asn

115

120

125

Ile Gln Cys Trp Leu Lys Gly Asp Leu Lys Leu Phe Ile Cys Tyr Val

130

135

140

Glu Ser Leu Phe Lys Asn Leu Phe Arg Asn Tyr Asn Tyr Lys Val His

145

150

155

160

Leu Leu Tyr Val Leu Pro Glu Val Leu Glu Asp Ser Pro Leu Val Pro

165

170

175

Gln Lys Gly Ser Phe Gln Met Val His Cys Asn Cys Ser Val His Glu

180

185

190

Cys Cys Glu Cys Leu Val Pro Val Pro Thr Ala Lys Leu Asn Asp Thr

195

200

205

Leu Leu Met Cys Leu Lys Ile Thr Ser Gly Gly Val Ile Phe Gln Ser

210

215

220

Pro Leu Met Ser Val Gln Pro Ile Asn Met Val Lys Pro Asp Pro Pro

225

230

235

240

Leu Gly Leu His Met Glu Ile Thr Asp Asp Gly Asn Leu Lys Ile Ser

245

250

255

Trp Ser Ser Pro Pro Leu Val Pro Phe Pro Leu Gln Tyr Gln Val Lys

260

265

270

Tyr Ser Glu Asn Ser Thr Thr Val Ile Arg Glu Ala Asp Lys Ile Val

275

280

285

Ser Ala Thr Ser Leu Leu Val Asp Ser Ile Leu Pro Gly Ser Ser Tyr

290

295

300

Glu Val Gln Val Arg Gly Lys Arg Leu Asp Gly Pro Gly Ile Trp Ser

305

310

315

320

Asp Trp Ser Thr Pro Arg Val Phe Thr Thr Gln Asp Val Ile Tyr Phe

325

330

335

Pro Pro Lys Ile Leu Thr Ser Val Gly Ser Asn Val Ser Phe His Cys

340

345

350

Ile Tyr Lys Lys Glu Asn Lys Ile Val Pro Ser Lys Glu Ile Val Trp

355

360

365

Trp Met Asn Leu Ala Glu Lys Ile Pro Gln Ser Gln Tyr Asp Val Val

370

375

380

Ser Asp His Val Ser Lys Val Thr Phe Phe Asn Leu Asn Glu Thr Lys

385

390

395

400

Pro Arg Gly Lys Phe Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu His

405

410

415

Glu Cys His His Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile

420	425	430
Asn Ile Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg		
435	440	445
Trp Ser Thr Ser Thr Ile Gln Ser Leu Ala Glu Ser Thr Leu Gln Leu		
450	455	460
Arg Tyr His Arg Ser Ser Leu Tyr Cys Ser Asp Ile Pro Ser Ile His		
465	470	475
480		
Pro Ile Ser Glu Pro Lys Asp Cys Tyr Leu Gln Ser Asp Gly Phe Tyr		
485	490	495
Glu Cys Ile Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp		
500	505	510
Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro Pro Thr Cys		
515	520	525
Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro Ser Ser Val Lys		
530	535	540
Ala Glu Ile Thr Ile Asn Ile Gly Leu Leu Lys Ile Ser Trp Glu Lys		
545	550	555
560		
Pro Val Phe Pro Glu Asn Asn Leu Gln Phe Gln Ile Arg Tyr Gly Leu		
565	570	575
Ser Gly Lys Glu Val Gln Trp Lys Met Tyr Glu Val Tyr Asp Ala Lys		
580	585	590

Ser Lys Ser Val Ser Leu Pro Val Pro Asp Leu Cys Ala Val Tyr Ala

595

600

605

Val Gln Val Arg Cys Lys Arg Leu Asp Gly Leu Gly Tyr Trp Ser Asn

610

615

620

Trp Ser Asn Pro Ala Tyr Thr Val Val Met Asp Ile Lys Val Pro Met

625

630

635

640

Arg Gly Pro Glu Phe Trp Arg Ile Ile Asn Gly Asp Thr Met Lys Lys

645

650

655

Glu Lys Asn Val Thr Leu Leu Trp Lys Pro Leu Met Lys Asn Asp Ser

660

665

670

Leu Cys Ser Val Gln Arg Tyr Val Ile Asn His His Thr Ser Cys Asn

675

680

685

Gly Thr Trp Ser Glu Asp Val Gly Asn His Thr Lys Phe Thr Phe Leu

690

695

700

Trp Thr Glu Gln Ala His Thr Val Thr Val Leu Ala Ile Asn Ser Ile

705

710

715

720

Gly Ala Ser Val Ala Asn Phe Asn Leu Thr Phe Ser Trp Pro Met Ser

725

730

735

Lys Val Asn Ile Val Gln Ser Leu Ser Ala Tyr Pro Leu Asn Ser Ser

740

745

750

Cys Val Ile Val Ser Trp Ile Leu Ser Pro Ser Asp Tyr Lys Leu Met

755

760

765

Tyr Phe Ile Ile Glu Trp Lys Asn Leu Asn Glu Asp Gly Glu Ile Lys

770

775

780

Trp Leu Arg Ile Ser Ser Ser Val Lys Lys Tyr Tyr Ile His Asp His

785

790

795

800

Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr Pro Ile Phe Met

805

810

815

Glu Gly Val Gly Lys Pro Lys Ile Ile Asn Ser Phe Thr Gln Asp Asp

820

825

830

Ile Glu Lys His Gln Ser Asp Ala Gly Leu Tyr Val Ile Val Pro Val

835

840

845

Ile Ile Ser Ser Ser Ile Leu Leu Leu Gly Thr Leu Leu Ile Ser His

850

855

860

Gln Arg Met Lys Lys Leu Phe Trp Glu Asp Val Pro Asn Pro Lys Asn

865

870

875

880

Cys Ser Trp Ala Gln Gly Leu Asn Phe Gln Lys Pro Glu Thr Phe Glu

885

890

895

His Leu Phe Ile Lys His Thr Ala Ser Val Thr Cys Gly Pro Leu Leu

900

905

910

Leu Glu Pro Glu Thr Ile Ser Glu Asp Ile Ser Val Asp Thr Ser Trp

915 920 925

Lys Asn Lys Asp Glu Met Met Pro Thr Thr Val Val Ser Leu Leu Ser
930 935 940

Thr Thr Asp Leu Glu Lys Gly Ser Val Cys Ile Ser Asp Gln Phe Asn
945 950 955 960

Ser Val Asn Phe Ser Glu Ala Glu Gly Thr Glu Val Thr Tyr Glu Ala
965 970 975

Glu Ser Gln Arg Gln Pro Phe Val Lys Tyr Ala Thr Leu Ile Ser Asn
980 985 990

Ser Lys Pro Ser Glu Thr Gly Glu Glu Gln Gly Leu Ile Asn Ser Ser
995 1000 1005

Val Thr Lys Cys Phe Ser Ser Lys Asn Ser Pro Leu Lys Asp Ser Phe
1010 1015 1020

Ser Asn Ser Ser Trp Glu Ile Glu Ala Gln Ala Phe Phe Ile Leu Ser
1025 1030 1035 1040

Asp Gln His Pro Asn Ile Ile Ser Pro His Leu Thr Phe Ser Glu Gly
1045 1050 1055

Leu Asp Glu Leu Leu Lys Leu Glu Gly Asn Phe Pro Glu Glu Asn Asn
1060 1065 1070

Asp Lys Lys Ser Ile Tyr Tyr Leu Gly Val Thr Ser Ile Lys Lys Arg
1075 1080 1085

Glu Ser Gly Val Leu Leu Thr Asp Lys Ser Arg Val Ser Cys Pro Phe

1090

1095

1100

Pro Ala Pro Cys Leu Phe Thr Asp Ile Arg Val Leu Gln Asp Ser Cys

1105

1110

1115

1120

Ser His Phe Val Glu Asn Asn Ile Asn Leu Gly Thr Ser Ser Lys Lys

1125

1130

1135

Thr Phe Ala Ser Tyr Met Pro Gln Phe Gln Thr Cys Ser Thr Gln Thr

1140

1145

1150

His Lys Ile Met Glu Asn Lys Met Cys Asp Leu Thr Val

1155

1160

1165

<210> 5

<211> 2229

<212> DNA

<213> Mus musculus

<400> 5

ggtagcccgcc ggagcctgag cgagcaggcg tccgtgcgga gccgaagacg ggaggaacat 60
gacatcgagg agatgggttc accccaacat cactgggtgtg gaggcagaga atctcctgct 120
gaccagagga gtcgatggca gtttttttagc aagggccagt aagagtaacc ctgggagactt 180
cactctgtct gttagaagaa atggagctgt taccacatc aagattcaga aactggggga 240
ctactatgac ctctatgggtg gggagaagtt tgccactttg gctgaactgg ttcagtatta 300
catggaacac catgggcagc tgaaagagaa gaatggagat gttatcgagc tcaagtaccc 360
gctgaactgt gcagacccta cctctgaaag gtggttccat ggtcacttgt ctggaaaaga 420
agcagagaag ctgctgacgg agaagggcaa gcatggcagc ttctctgttc gagagagcca 480

gagccacccc ggagacttcg ttctctccgt gcgcactggg gagacaaaag gggagagcaa 540
cgacggcaag tccaaagtga cccacgtcat gatccgctgt caggagctga aatacgacgt 600
tggtggggga gagcgctttg actctctgac agacctgggt gagcattaca agaagaaccc 660
catggtggag acgctgggca cagtccctgca gctcaagcag cccctcaaca caactcgtat 720
caatgctgct gaaattgaaa gccgggttcg agagttaagc aagctggctg agaccacaga 780
taaagtcaag cagggcctttt ggaagagtt tgagacgctc cagcaacagg aatgcaaact 840
tctctatagc cgaaaagaag gacagagaca agaaaataaa aacaaaaaca gatacaaaaa 900
catcctgccc ttgatcata ccagggtcgt tctgcatgat ggggatccca atgagcctgt 960
ttctgattac attaatgcaa acatcatcat gcctgagttt gagaccaagt gcaacaattc 1020
caaaccctaa aagagttaca ttgccactca aggtgcctg cagaacacgg tgaatgactt 1080
ctggcgggatg gtgttccagg agaactctcg agtcattgtc atgaccacaa aggaagtggg 1140
gagaggggaag agcaaagtgt tcaagtactg gcctgatgag tatgcgctca agaatacgg 1200
ggatcatgctg gttaggaacg tcaaagaaag tgccgcccac gactacactt tacgagagct 1260
caaactctct aaggtcggac aagctctact ccagggaac acagagagaa ccgtctggca 1320
gtaccacttt cggacctggc cagaccatgg cgtgcctagt gacctggag gtgtgctgga 1380
cttctctggag gaggtccacc acaagcagga gagcatcgtg gatgcaggcc ctgtcgtggt 1440
tactgcagc gctgggattg gccggacagg aaccttcatt gtgattgaca tccttattga 1500
catcattcga gagaaagggt tggactgtga catcgacgtt cctaaaacca ttcagatggt 1560
gcgggtcccag aggtcgggga tggccagac agaagcacag taccggttta tctacatggc 1620
tgtccagcac tacatagaga cgctgcagcg ccggatcgag gaggagcaga aaagcaaaag 1680
aaaaggacat gaatatacca atattaagta ttccttgggt gaccagacaa gtggtgatca 1740
gagtcctctg ccacctgca cccaacgcc accctgtgca gaaatgaggg aggacagcgc 1800
ccgagtctat gagaacgtgg gcctcatgca gcagcagagg agtttcagat gagcccacgg 1860
cgacagaacg cagatgtgaa ctttcacccc tttcctaaaa tgtcaagact agacgagcgt 1920
tcccaggacc cgtgcgtgcg ttcagaagcc ggcactggct ggactgcctc ttgagaagcg 1980
aagtttggaa ccatttgaac agcacgtgcc taattggcac ctcttttct cagctaagga 2040
gagactgctc tgcgttcttg acaatgctat tttcatagaa ttggttttga attgtggaag 2100
cagctaaatt gtgctctgta ttttctacat tatgggactc aaattctagt tatgggcagg 2160
atattgtttc tttttatgac cttaacagat ctgatttttt ttttctttct ctctctttgg 2220
ggaatcatg 2229

<210> 6

<211> 597

<212> PRT

<213> Mus musculus

<400> 6

Met Thr Ser Arg Arg Trp Phe His Pro Asn Ile Thr Gly Val Glu Ala

1 5 10 15

Glu Asn Leu Leu Leu Thr Arg Gly Val Asp Gly Ser Phe Leu Ala Arg

20 25 30

Pro Ser Lys Ser Asn Pro Gly Asp Phe Thr Leu Ser Val Arg Arg Asn

35 40 45

Gly Ala Val Thr His Ile Lys Ile Gln Asn Thr Gly Asp Tyr Tyr Asp

50 55 60

Leu Tyr Gly Gly Glu Lys Phe Ala Thr Leu Ala Glu Leu Val Gln Tyr

65 70 75 80

Tyr Met Glu His His Gly Gln Leu Lys Glu Lys Asn Gly Asp Val Ile

85 90 95

Glu Leu Lys Tyr Pro Leu Asn Cys Ala Asp Pro Thr Ser Glu Arg Trp

100 105 110

Phe His Gly His Leu Ser Gly Lys Glu Ala Glu Lys Leu Leu Thr Glu

115 120 125

Lys Gly Lys His Gly Ser Phe Leu Val Arg Glu Ser Gln Ser His Pro

130 135 140
Gly Asp Phe Val Leu Ser Val Arg Thr Gly Asp Asp Lys Gly Glu Ser
145 150 155 160
Asn Asp Gly Lys Ser Lys Val Thr His Val Met Ile Arg Cys Gln Glu
165 170 175
Leu Lys Tyr Asp Val Gly Gly Gly Glu Arg Phe Asp Ser Leu Thr Asp
180 185 190
Leu Val Glu His Tyr Lys Lys Asn Pro Met Val Glu Thr Leu Gly Thr
195 200 205
Val Leu Gln Leu Lys Gln Pro Leu Asn Thr Thr Arg Ile Asn Ala Ala
210 215 220
Glu Ile Glu Ser Arg Val Arg Glu Leu Ser Lys Leu Ala Glu Thr Thr
225 230 235 240
Asp Lys Val Lys Gln Gly Phe Trp Glu Glu Phe Glu Thr Leu Gln Gln
245 250 255
Gln Glu Cys Lys Leu Leu Tyr Ser Arg Lys Glu Gly Gln Arg Gln Glu
260 265 270
Asn Lys Asn Lys Asn Arg Tyr Lys Asn Ile Leu Pro Phe Asp His Thr
275 280 285
Arg Val Val Leu His Asp Gly Asp Pro Asn Glu Pro Val Ser Asp Tyr
290 295 300

Ile Asn Ala Asn Ile Ile Met Pro Glu Phe Glu Thr Lys Cys Asn Asn
305 310 315 320

Ser Lys Pro Lys Lys Ser Tyr Ile Ala Thr Gln Gly Cys Leu Gln Asn
325 330 335

Thr Val Asn Asp Phe Trp Arg Met Val Phe Gln Glu Asn Ser Arg Val
340 345 350

Ile Val Met Thr Thr Lys Glu Val Glu Arg Gly Lys Ser Lys Cys Val
355 360 365

Lys Tyr Trp Pro Asp Glu Tyr Ala Leu Lys Glu Tyr Gly Val Met Arg
370 375 380

Val Arg Asn Val Lys Glu Ser Ala Ala His Asp Tyr Thr Leu Arg Glu
385 390 395 400

Leu Lys Leu Ser Lys Val Gly Gln Ala Leu Leu Gln Gly Asn Thr Glu
405 410 415

Arg Thr Val Trp Gln Tyr His Phe Arg Thr Trp Pro Asp His Gly Val
420 425 430

Pro Ser Asp Pro Gly Gly Val Leu Asp Phe Leu Glu Glu Val His His
435 440 445

Lys Gln Glu Ser Ile Val Asp Ala Gly Pro Val Val Val His Cys Ser
450 455 460

Ala Gly Ile Gly Arg Thr Gly Thr Phe Ile Val Ile Asp Ile Leu Ile

465 470 475 480

Asp Ile Ile Arg Glu Lys Gly Val Asp Cys Asp Ile Asp Val Pro Lys

485 490 495

Thr Ile Gln Met Val Arg Ser Gln Arg Ser Gly Met Val Gln Thr Glu

500 505 510

Ala Gln Tyr Arg Phe Ile Tyr Met Ala Val Gln His Tyr Ile Glu Thr

515 520 525

Leu Gln Arg Arg Ile Glu Glu Glu Gln Lys Ser Lys Arg Lys Gly His

530 535 540

Glu Tyr Thr Asn Ile Lys Tyr Ser Leu Val Asp Gln Thr Ser Gly Asp

545 550 555 560

Gln Ser Pro Leu Pro Pro Cys Thr Pro Thr Pro Pro Cys Ala Glu Met

565 570 575

Arg Glu Asp Ser Ala Arg Val Tyr Glu Asn Val Gly Leu Met Gln Gln

580 585 590

Gln Arg Ser Phe Arg

595

<210> 7

<211> 1980

<212> DNA

<213> Homo sapiens

<400> 7

ggcacgagcg gctggctctg cccgcgtccg gtcccagagcg ggctccctc gggccagccc 60
gatgtgaccg agcccagcgg agcctgagca aggagcgggt cgcgcgga gccggagggc 120
gggaggaaca tgacatcgcg gagatggttt caccxaaata tcaactgggtg ggaggcagaa 180
aacctactgt tgacaagagg agttgatggc agttttttgg caaggcctag taaaagtaac 240
cctggagact tcacactttc cgttagaaga aatggagctg tcaccacat caagattcag 300
aacactgggtg attactatga cctgtatgga ggggagaaat ttgccacttt ggctgagttg 360
gtccagtatt acatggaaca tcacgggcaa ttaaagaga agaattggaga tgcattgag 420
ctaaatatc ctctgaactg tgcagatcct acctctgaaa ggtgggttca tggacatctc 480
tctgggaaag aagcagagaa attattaact gaaaaaggaa aacatggtag ttttcttgta 540
cgagagagcc agagccaccc tggagatttt gttctttctg tgcgcactgg tgatgacaaa 600
ggggagagca atgacggcaa gtctaaagt acccatgtta tgattcgctg tcaggaactg 660
aaatacgacg ttggtggagg agaacggttt gattctttga cagatcttgt ggaacattat 720
aagaagaatc ctatgggtgga aacattgggt acagtactac aactcaagca gcccctaac 780
acgactcgta taaatgctgc tgaaatagaa agcagagttc gagaactaag caaattagct 840
gagaccacag ataaagtcaa acaaggcttt tgggaagaat ttgagacact acaacaacag 900
gagtgc aaac ttctctacag ccgaaaagag ggtcaaaggc aagaaaacaa aaacaaaaat 960
agatataaaa acatcctgcc ctttgatcat accaggggtg tcctacacga tgggtgatccc 1020
aatgagcctg tttcagatta catcaatgca aatatcatca tgcctgaatt tgaaaccaag 1080
tgcaacaatt caaagcccaa aaagagttac attgccacac aaggctgcct gcaaaacacg 1140
gtgaatgact tttggcggat ggtgttccaa gaaaactccc gagtgattgt catgacaacg 1200
aaagaagtgg agagaggaaa gagtaaagt gtcaaatact ggctgatga gtatgctcta 1260
aaagaatatg gcgtcatgcg tgttaggaac gtcaaagaaa gcgcgctca tgactatacg 1320
ctaagagaac ttaactttc aaagggttga caagggaata cgagagaaac ggtctggcaa 1380
taccactttc ggacctggcc ggaccacggc gtgccagcg accctggggg cgtgctggac 1440
ttctggagg aggtgcacca taagcaggag agcatcatgg atgcagggcc ggtcgtggtg 1500
cactgcagtg ctggaattgg ccggacaggg acgttcattg tgattgatat tcttattgac 1560
atcatcagag agaaaggtgt tgactgcat attgacgttc caaaacat ccagatgggtg 1620
cggtctcaga ggtcagggt ggtccagaca gaagcacagt accgatttat ctatatggcg 1680

gtccagcatt atattgaaac actacagcgc aggattgaag aagagcagaa aagcaagagg 1740
 aaagggcacg aatatacaaa tattaagtat tctctagcgg accagacgag tggagatcag 1800
 agccctctcc cgccttgtac tccaacgcca ccctgtgcag aaatgagaga agacagtgct 1860
 agagtctatg aaaacgtggg cctgatgcaa cagcagaaaa gtttcagatg agaaaacctg 1920
 ccaaaacttc agcacagaaa tagatgtgga ctttcacctc tccctaaaaa gatcaggacc 1980

<210> 8

<211> 593

<212> PRT

<213> Homo sapiens

<400> 8

Met Thr Ser Arg Arg Trp Phe His Pro Asn Ile Thr Gly Val Glu Ala

1 5 10 15

Glu Asn Leu Leu Leu Thr Arg Gly Val Asp Gly Ser Phe Leu Ala Arg

20 25 30

Pro Ser Lys Ser Asn Pro Gly Asp Phe Thr Leu Ser Val Arg Arg Asn

35 40 45

Gly Ala Val Thr His Ile Lys Ile Gln Asn Thr Gly Asp Tyr Tyr Asp

50 55 60

Leu Tyr Gly Gly Glu Lys Phe Ala Thr Leu Ala Glu Leu Val Gln Tyr

65 70 75 80

Tyr Met Glu His His Gly Gln Leu Lys Glu Lys Asn Gly Asp Val Ile

85 90 95

Glu Leu Lys Tyr Pro Leu Asn Cys Ala Asp Pro Thr Ser Glu Arg Trp

100 105 110
Phe His Gly His Leu Ser Gly Lys Glu Ala Glu Lys Leu Leu Thr Glu
115 120 125
Lys Gly Lys His Gly Ser Phe Leu Val Arg Glu Ser Gln Ser His Pro
130 135 140
Gly Asp Phe Val Leu Ser Val Arg Thr Gly Asp Asp Lys Gly Glu Ser
145 150 155 160
Asn Asp Gly Lys Ser Lys Val Thr His Val Met Ile Arg Cys Gln Glu
165 170 175
Leu Lys Tyr Asp Val Gly Gly Gly Glu Arg Phe Asp Ser Leu Thr Asp
180 185 190
Leu Val Glu His Tyr Lys Lys Asn Pro Met Val Glu Thr Leu Gly Thr
195 200 205
Val Leu Gln Leu Lys Gln Pro Leu Asn Thr Thr Arg Ile Asn Ala Ala
210 215 220
Glu Ile Glu Ser Arg Val Arg Glu Leu Ser Lys Leu Ala Glu Thr Thr
225 230 235 240
Asp Lys Val Lys Gln Gly Phe Trp Glu Glu Phe Glu Thr Leu Gln Gln
245 250 255
Gln Glu Cys Lys Leu Leu Tyr Ser Arg Lys Glu Gly Gln Arg Gln Glu
260 265 270

Asn Lys Asn Lys Asn Arg Tyr Lys Asn Ile Leu Pro Phe Asp His Thr
275 280 285

Arg Val Val Leu His Asp Gly Asp Pro Asn Glu Pro Val Ser Asp Tyr
290 295 300

Ile Asn Ala Asn Ile Ile Met Pro Glu Phe Glu Thr Lys Cys Asn Asn
305 310 315 320

Ser Lys Pro Lys Lys Ser Tyr Ile Ala Thr Gln Gly Cys Leu Gln Asn
325 330 335

Thr Val Asn Asp Phe Trp Arg Met Val Phe Gln Glu Asn Ser Arg Val
340 345 350

Ile Val Met Thr Thr Lys Glu Val Glu Arg Gly Lys Ser Lys Cys Val
355 360 365

Lys Tyr Trp Pro Asp Glu Tyr Ala Leu Lys Glu Tyr Gly Val Met Arg
370 375 380

Val Arg Asn Val Lys Glu Ser Ala Ala His Asp Tyr Thr Leu Arg Glu
385 390 395 400

Leu Lys Leu Ser Lys Val Gly Gln Gly Asn Thr Glu Arg Thr Val Trp
405 410 415

Gln Tyr His Phe Arg Thr Trp Pro Asp His Gly Val Pro Ser Asp Pro
420 425 430

Gly Gly Val Leu Asp Phe Leu Glu Glu Val His His Lys Gln Glu Ser

435

440

445

Ile Met Asp Ala Gly Pro Val Val Val His Cys Ser Ala Gly Ile Gly

450

455

460

Arg Thr Gly Thr Phe Ile Val Ile Asp Ile Leu Ile Asp Ile Ile Arg

465

470

475

480

Glu Lys Gly Val Asp Cys Asp Ile Asp Val Pro Lys Thr Ile Gln Met

485

490

495

Val Arg Ser Gln Arg Ser Gly Met Val Gln Thr Glu Ala Gln Tyr Arg

500

505

510

Phe Ile Tyr Met Ala Val Gln His Tyr Ile Glu Thr Leu Gln Arg Arg

515

520

525

Ile Glu Glu Glu Gln Lys Ser Lys Arg Lys Gly His Glu Tyr Thr Asn

530

535

540

Ile Lys Tyr Ser Leu Ala Asp Gln Thr Ser Gly Asp Gln Ser Pro Leu

545

550

555

560

Pro Pro Cys Thr Pro Thr Pro Pro Cys Ala Glu Met Arg Glu Asp Ser

565

570

575

Ala Arg Val Tyr Glu Asn Val Gly Leu Met Gln Gln Gln Lys Ser Phe

580

585

590

Arg

Tyr Phosphorylated Ob-Rb Cytoplasmic Constructs

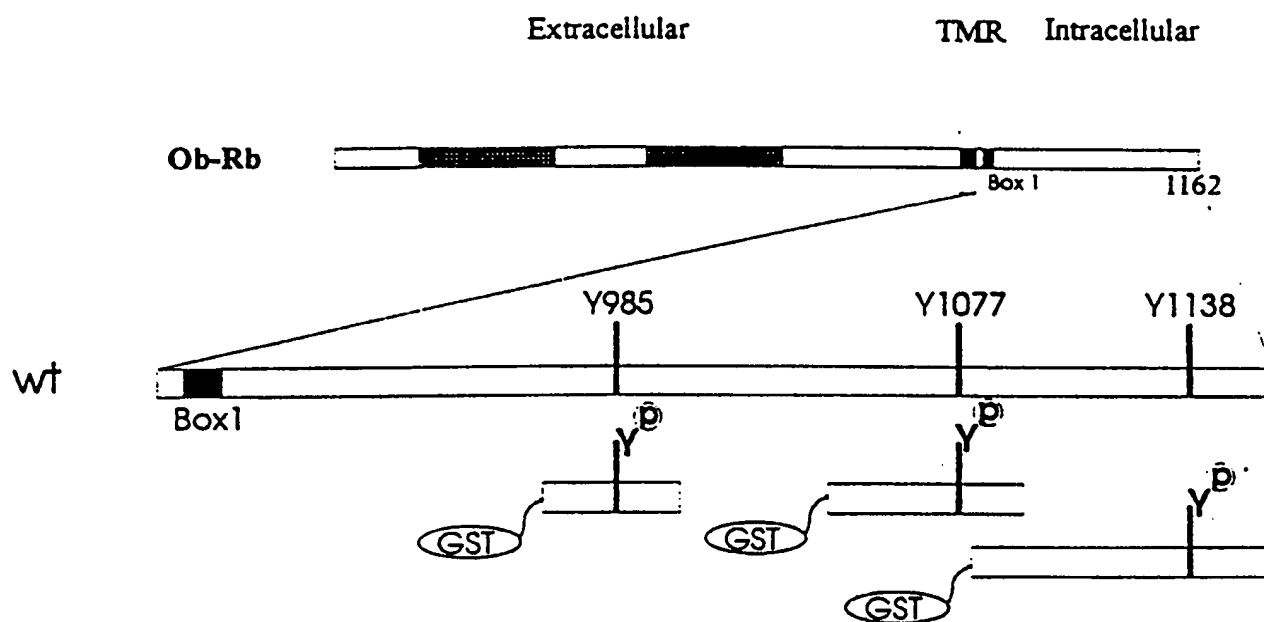


Figure 1A

Binding of GST-Y1[®] to PTP1D

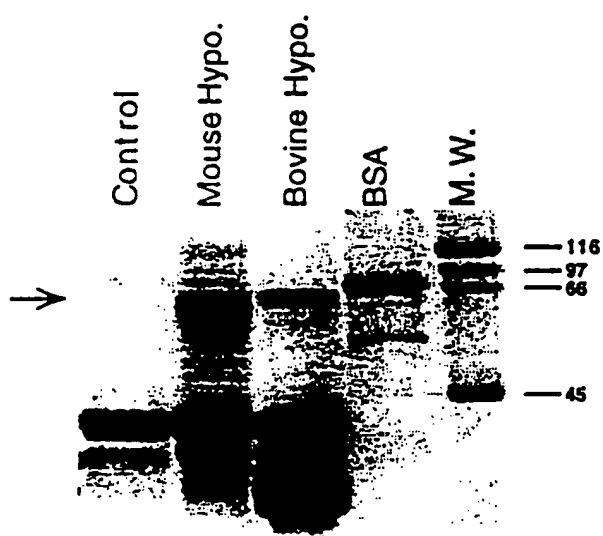


Figure 1B

Immunoblot of Hypothalamic Precipitates

A.

GST-ObRb Tyr 1

-P +P

-

PIP₂D

B.

GST-ObRb Tyr 3

-P +P

-

stat 3

Figure 2

Involvement of Jak2 in Ob-Rb dependent PTP1D Activation

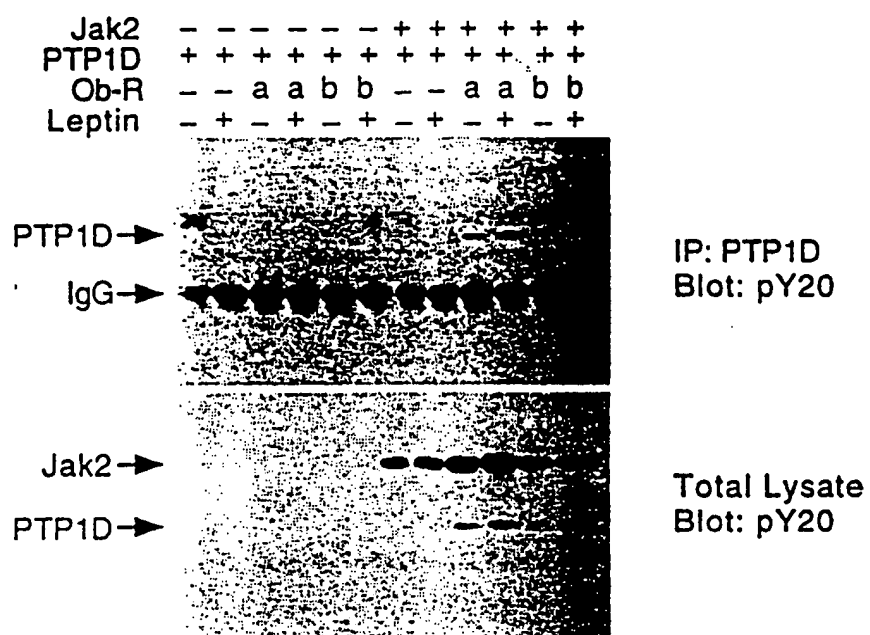


Figure 3

Time Course of Ob-Rb dependent PTP1D Activation

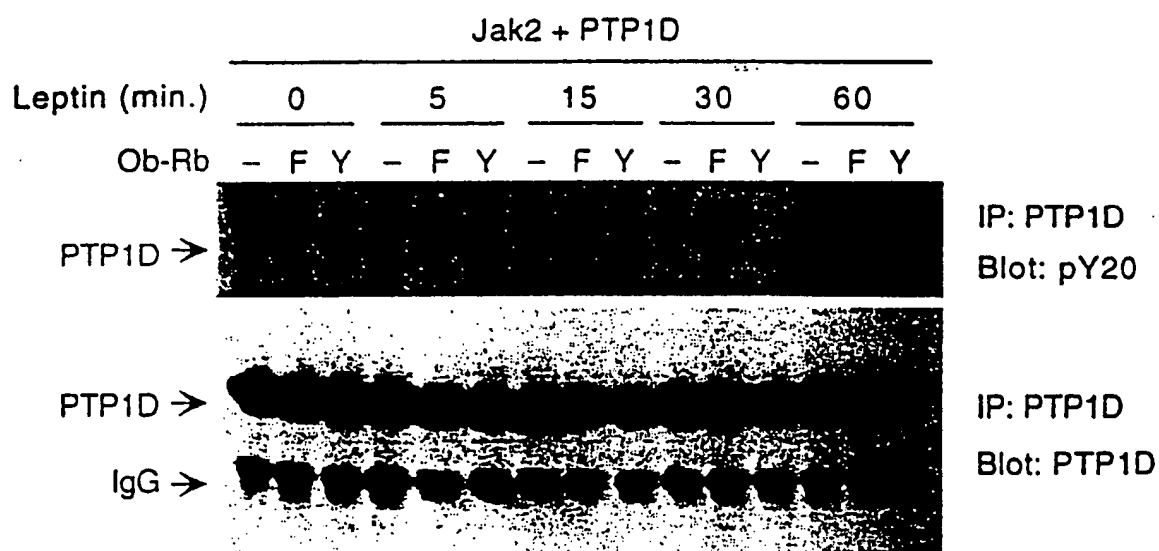


Figure 4

6/6

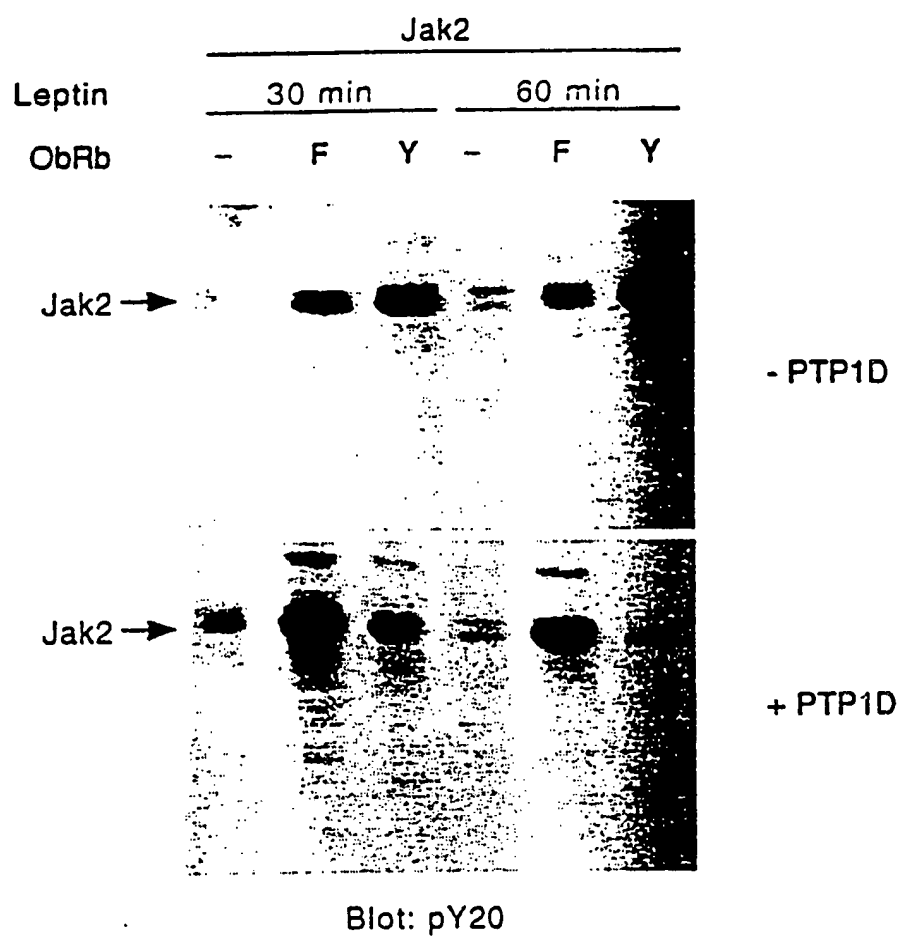


Figure 5

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/22797

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G01N33/68 G01N33/573 G01N33/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 26523 A (PROGENITOR INCORPORATED) 24 July 1997 see page 2, line 8 - line 9; claim 11 ---	1-20
A	WO 96 22308 A (ZYMOGENETICS INC & UNIVERSITY OF WASHINGTON) 25 July 1996 cited in the application see claim 1 ---	16,20
A	WO 96 38586 A (SMITHKLINE BEECHAM PLC) 5 December 1996 see the whole document ---	1-20
A	WO 94 08017 A (MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V.) 14 April 1994 see the whole document -----	1-20



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

3 March 1999

Date of mailing of the international search report

10/03/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Van Bohemen, C

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internal Application No

PGT/US 98/22797

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9726523 A	24-07-1997	AU 1833197 A	11-08-1997
WO 9622308 A	25-07-1996	US 5827734 A	27-10-1998
		AU 4863196 A	07-08-1998
		US 5858967 A	12-01-1999
WO 9638586 A	05-12-1996	AU 6002396 A	18-12-1996
		CA 2222409 A	05-12-1996
		CN 1192248 A	02-09-1998
		CZ 9703790 A	13-05-1998
		EP 0832284 A	01-04-1998
		HU 9801744 A	28-10-1998
		NO 975504 A	28-11-1997
		PL 323638 A	14-04-1998
WO 9408017 A	14-04-1994	US 5589375 A	31-12-1996
		AU 5175793 A	26-04-1994
		CA 2146544 A	14-04-1994
		CN 1091469 A	31-08-1994
		EP 0663954 A	26-07-1995
		JP 8502885 T	02-04-1996
		MX 9306220 A	31-05-1996